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(71) Applicant: **PLANT GENETIC SYSTEMS, N.V.**
Kolonel Bourgstraat 106 Bus 6
B-1040 Brussel(BE)

(72) Inventor: **De Greef, Willy**
Coupure Rechts 154
B-9000 Gent(BE)
Inventor: **Van Emmelo, John**
Gerardstraat 8
B-9110 Sint-Amandsberg(BE)
Inventor: **De Oliveira, Dulce Eleonora**
Av. Lineu de Paula Machado 905/507
20470 Lagoa, BR- Rio de Janeiro(BR)
Inventor: **De Souza, Maria-Helena**
Holdaal 31
B-9000 Gent(BE)
Inventor: **Van Montagu, Marc**
De Stassartstraat 120
B-1050 Brussels(BE)

(74) Representative: **Gutmann, Ernest et al**
S.C. Ernest Gutmann - Yves Plasseraud 67, boulevard Haussmann
F-75008 Paris(FR)

(54) Plants with modified flowers, seeds or embryos.

(57) A plant, the nuclear genome of which is transformed with a foreign DNA sequence encoding a product which selectively disrupts the metabolism, functioning and/or development of cells of the flowers, particularly one or more of their female organs, or the seeds or the embryos of the plant. The foreign DNA sequence also optionally encodes a marker.

EP 0 412 006 A1

PLANTS WITH MODIFIED FLOWERS, SEEDS OR EMBRYOS

This invention relates to a female-sterile plant and to its reproductive material (e.g., seeds), in which the cells have been transformed so that a foreign DNA sequence is stably integrated into their nuclear genome. The foreign DNA sequence of this invention contains a first foreign DNA (hereinafter the "female-sterility DNA") that: 1) encodes a first RNA, protein or polypeptide which, when produced or overproduced in a cell of a flower, particularly a female organ thereof, or a seed or an embryo of a plant, disturbs significantly the metabolism, functioning and/or development of the cell of the flower or seed or embryo; and 2) is in the same transcriptional unit as, and under the control of, a first promoter which is capable of directing expression of the female-sterility DNA selectively in cells of the flowers, particularly one or more of their female organs, or seeds or embryos of the plant. In particular, this invention relates to such a nuclear female-sterile plant and its reproductive material, in which the foreign DNA sequence of this invention is a foreign chimaeric DNA sequence that can also contain a second foreign DNA (the "marker DNA") that: encodes a second RNA, protein or polypeptide which, when present at least in a specific tissue or specific cells of the plant, renders the entire plant easily separable from other plants that do not contain the second RNA, protein or polypeptide at least in the specific tissue or specific cells; 2) is in the same transcriptional unit as, and under the control of, a second promoter which is capable of directing expression of the marker DNA in at least the specific tissue or the specific cells of the plant; and 3) is in the same genetic locus of the nuclear genome of the cells of the plant as the female-sterility DNA.

This invention also relates to a foreign chimaeric DNA sequence that contains at least one female-sterility DNA under the control of at least one first promoter and that can also contain, adjacent to the female-sterility DNA(s) and the first promoter(s), at least one marker DNA under the control of at least one second promoter.

This invention further relates to a vector that contains the foreign DNA sequence of this invention and is suitable for the transformation of a plant cell, whereby the foreign DNA sequence is stably integrated into the nuclear genome of the cell.

This invention still further relates to cells of a plant and to plant cell cultures, the nuclear genomes of which are transformed with the foreign DNA sequence.

This invention yet further relates to a process for producing a nuclear female-sterile male-fertile plant and its reproductive material containing the foreign DNA sequence in which the female-sterility DNA: 1) is under the control of the first promoter; 2) is stably integrated into the nuclear genome of the plant's cells; 3) can be expressed selectively in cells of each flower, particularly a female organ thereof, or each seed or each embryo of the plant in the form of the first RNA, protein or polypeptide; and optionally 4) is in the same genetic locus as the marker DNA under the control of the second promoter.

The invention further relates to a process for producing hybrid seeds, which grow into hybrid plants, by crossing: 1) the female-sterile plant of this invention which may include, in its nuclear genome, the marker DNA, preferably encoding a protein conferring a resistance to a herbicide on the plant; and 2) a female-fertile plant without the marker DNA in its genome. This invention particularly relates to such a process for producing hybrid seeds on a commercial scale, preferably in a substantially random population, without the need for extensive hand-labor.

Background of the Invention

Hybridization of plants is recognized as an important process for producing offspring having a combination of the desirable traits of the parent plants. The resulting hybrid offspring often has the ability to outperform the parents in different traits, such as in yield, adaptability to environmental changes, and disease resistance. This ability is called "heterosis" or "hybrid vigor". As a result, hybridization has been used extensively for improving major crops, such as corn, sugar beet and sunflower. For a number of reasons, primarily related to the fact that most plants are capable of undergoing both self-pollination and cross-pollination, the controlled cross-pollination of plants without significant self-pollination, to produce a harvest of hybrid seeds, has been difficult to achieve on a commercial scale.

In nature, the vast majority of crop plants produce male and female reproductive organs on the same plant, usually in close proximity to one another in the same flower. This favors self-pollination. Some plants, however, are exceptions as a result of the particular morphology of their reproductive organs which favors cross-pollination. These plants produce hybrid offspring with improved vigor and adaptability. One such morphology in Cannabis ssp. (hemp) involves male and female reproductive organs on separate plants.

Another such morphology in Zea mays (corn) involves male and female reproductive organs on different parts of the same plant. Another such morphology in Elaeis guineensis (oil palm) involves male and fertile female gametes which become fertile at different times in the plant's development.

Some other plant species, such as Ananas comosus (pineapple), favor cross-pollination through the particular physiology of their reproductive organs. Such plants have developed a so-called "self-incompatibility system" whereby the pollen of one plant is not able to fertilize the female gamete of the same plant or of another plant with the same genotype.

Some other plant species favor cross-pollination by naturally displaying the so-called genomic characteristic of "male sterility". By this characteristic, the plants anthers degenerate before pollen, produced by the anthers, reaches maturity. See: "Male-Sterility in Higher Plants", M.L.H. Kaul, 1987, in: Monographs on Theoretical and Applied Genetics 10, Edit. Springer Verlag. Such a natural male-sterility characteristic is believed to result from a wide range of natural mutations, most often involving recessive deficiencies, and this characteristic can not easily be maintained in plant species that predominantly self-pollinate, since under natural conditions, no seeds will be produced.

Some other plants favor cross-pollination by natually displaying the character of "female-sterility" due to a deficient functioning of either the female gametophyte, the female gamete, the female zygote, or the seed. These plants produce no viable seeds. There are many different mutations that can lead to this condition, involving all stages of development of a specific tissue of the female reproductive organ. This characteristic distinguishes female-sterility from the more widely known phenomena of male-sterility and self-incompatibility. Although reducing the number of offspring a species can produce, the female-sterility trait has some evolutionary advantages in nature for some plants, especially for perennials. In perennials, the rate of vegetative growth is to a large extend determined by the distribution of biomass between vegetative and reproductive plant tissues. Female-sterile plants therefore tend to grow more vigorously than the female-fertile plants.

Although female-sterility inducing mutations probably occur as frequently as male-sterility inducing mutations, female-sterility inducing mutations are much less used in plant breeding and seed production and consequently much less studied, and only few examples of such mutations exist.

A well documented illustration of natural female-sterility is the oil palm (Elaeis guineensis) where the so-called "pisifera" condition is characterized by the inability of the developing seed to produce a shell. As a result, the developing seed aborts in an early stage, and no ripe fruit is formed. The gene encoding the "pisifera" genotype acts as a semi-dominant allele. Plants homozygous for the allele are not capable of producing a seed shell and consequently no ripe fruit or seeds. Plants heterozygotic for the allele produce ripe fruit and seeds with a thin shell (0.5 to 2 mm), while wild-type plants (which do not carry the allele) produce ripe fruit and seeds with shells of 2 to 6 mm thickness. These two genotypes are indistinguishable in seed yield, and their genotype is determined by that of the female parent plant. In oil palm breeding, the "pisifera" type is used as the male parent plant in all commercial seed production. By crossing pollen from the "pisifera" palms with the wild-type female parent plants, a homogeneous F1 hybrid seed population, producing thin-shelled fruit, is obtained.

Another example of a plant with a natural female-sterility, used for the commercial production of hybrid seed, is alfalfa. Alfalfa was known to have male-sterility genes, but in testing a hybrid seed production system in which male-sterile and male fertile plants were sown in separate bands, it appeared that a negligeable amount of hybrid seeds was produced. This low production was due to the fact that honeybees, responsible for pollination, have low affinity for male-sterile plants, favoring the self-pollination of the male-fertile plants. To obtain good seed set, it seemed necessary to interplant very closely to each other (thus not in separate rows) the male-fertile and the male-sterile parent plants. This was made possible when a female-sterility gene was discovered and bred into the male-fertile plants. Consequently, the only seeds which could be produced in the randomly sown plots were hybrid seeds obtained by cross-pollination between the female-sterile and the male-sterile parent plants.

For other crops, female-sterility has been reported, such as sorghum (Casady et al (1960) J. Hered. 51 , 35-38), cotton (Tustus and Meyer (1963) J. Hered. 54 , 167-168), tomato (Honma and Pratak (1964) J. Hered. 55 , 143-145), wheat (Gotzov and Dzelepov (1974) Gen. Plant Breed. 7, 480-487), and pearl millet (Hanna and Powel (1974) J. Hered. 65 , 247-249). There are, however, several problems in maintaining the female-sterile lines, and for this reason, such lines are not used on a commercially important scale.

Compared with male-sterility, the use of female-sterility offers some other advantages in the production of hybrid seeds. Female-sterility allows the production of fruits without seeds and enhanced vegetative biomass production and can, in some cases, induce more flower-setting within one season.

Summary of the Invention

In accordance with this invention, a cell of a plant is provided, in which the nuclear genome contains, stably integrated therein, a foreign DNA sequence, preferably a foreign chimaeric DNA sequence, characterized by:

(a) a female-sterility DNA encoding a first RNA, protein or polypeptide which, when produced or overproduced in a cell of a flower, particularly a female organ thereof, a seed or an embryo of the plant, disturbs significantly the metabolism, functioning and/or development of the cell of the flower or seed or embryo; and

(b) a first promoter capable of directing expression of the female-sterility DNA selectively in cells of the flowers, particularly a female organ thereof, seeds or embryos of the plant; the female-sterility DNA being in the same transcriptional unit as, and under the control of, the first promoter.

The foreign DNA sequence in the nuclear genome of the transformed cell can also comprise, preferably in the same genetic locus as the female-sterility DNA:

(c) a marker DNA encoding a second RNA, protein or polypeptide which, when present at least in a specific tissue or specific cells of the plant, renders the plant easily separable from other plants which do not contain the second RNA, protein or polypeptide at least in the specific tissue or specific cells; and

(d) a second promoter capable of directing expression of the marker DNA at least in the specific tissue or specific cells; the marker DNA being in the same transcriptional unit as, and under the control of, the second promoter.

Also in accordance with this invention is provided a foreign chimaeric DNA sequence that comprises the female-sterility DNA and the first promoter and that can also comprise the marker DNA and the second promoter, as well as at least one additional DNA encoding: a transit peptide capable of transporting the first protein or polypeptide and/or the second protein or polypeptide into a chloroplast or mitochondria of a plant cell, in which the foreign chimaeric DNA sequence is expressed in its cytoplasm; and/or a secretory signal peptide capable of secreting the first protein or polypeptide and/or the second protein or polypeptide out of a plant cell or plant tissue, in which the foreign chimaeric DNA sequence is expressed.

Further in accordance with this invention are provided: a female-sterile male-fertile plant and a plant cell culture, each consisting of cells containing the foreign DNA sequence; a fruit of a female-sterile plant; hybrid seeds and plants produced by crossing the female-sterile plant with a female-fertile plant; and a process for producing such hybrid seeds, as well as seedless fruit.

Yet further in accordance with this invention are provided style-, stigma-, ovary-, seed- and embryo-specific first promoters.

Detailed Description of the Invention

In accordance with this invention, a female-sterile male-fertile plant is produced from a single cell of a plant by transforming the plant cell in a well known manner to stably insert, into the nuclear genome of the cell, the foreign DNA sequence of this invention. The foreign DNA sequence comprises at least one female-sterility DNA that is under the control of, and fused at its 5′ end to, the first promoter and is fused at its 3′ end to suitable transcription termination (or regulation) signals, including a polyadenylation signal. Thereby, the first RNA, protein or polypeptide is produced or overproduced selectively in cells of all the flowers, particularly in one or more female organs thereof, and/or in all the seeds and/or in all the embryos of the plant so as to render the plant female-sterile. The foreign DNA sequence can also comprise at least one marker DNA that is under the control of, and is fused at its 5′ end to, the second promoter and is fused at its 3′ end to suitable transcription termination signals, including a polyadenylation signal. The marker DNA is preferably in the same genetic locus as the female-sterility DNA, whereby the second RNA, protein or polypeptide is produced in at least the specific tissue or specific cells of the female-sterile plant so that the plant can be easily distinguished and/or separated from other plants that do not contain the second RNA, protein or polypeptide in the specific tissue or specific cells. This guarantees, with a high degree of certainty, the joint segregation of both the female-sterility DNA and the marker DNA into offspring of the plant.

The cell of the plant (particularly a plant capable of being infected with Agrobacterium) is preferably transformed in accordance with this invention, using a vector that is a disarmed Ti-plasmid containing the foreign DNA sequence and carried by Agrobacterium . This transformation can be carried out using procedures described, for example, in European patent publications 0,116,718 and 0,270,822. Preferred Ti-plasmid vectors contain the foreign DNA sequence between the border sequences, or at least located to the

left of the right border sequence, of the T-DNA of the Ti-plasmid. Of course, other types of vectors can be used to transform the plant cell, using procedures such as direct gene transfer (as described, for example, in European patent publication 0,223,247), pollen mediated transformation (as described, for example, in European patent publication 0,270,356, PCT publication WO85/01856, and European patent publication 0,275,069), in vitro protoplast transformation (as described, for example, in US patent 4,684,611), plant RNA virus-mediated transformation (as described, for example, in European patent publication 0,067,553 and US patent 4,407,956) and liposome-mediated transformation (as described, for example, in US patent 4,536,475).

Preferably, a nuclear female-sterile male-fertile plant of this invention is provided by transforming a plant cell with a disarmed Ti-plasmid vector containing the foreign DNA sequence with a female-sterility DNA under the control of a first promoter and preferably a marker DNA under the control of a second promoter. The marker DNA can be upstream or downstream of the female-sterility DNA in the Ti-plasmid vector, but preferably, the two are adjacent to one another and are located between the border sequences or at least located to the left of the right border sequence of the Ti-plasmid vector, so that they are properly transferred together into the nuclear genome of the plant cell. However, if desired, the cell can initially be transformed with a foreign DNA sequence containing a female-sterility DNA and a first promoter and can subsequently be transformed with a marker DNA and a second promoter, inserted into or near the genetic locus of the female-sterility DNA in the cell's nuclear genome, or this transformation can be carried out vice versa. Suitable vectors for this purpose are the same as those discussed above for transforming cells with the foreign DNA sequence. The preferred vector is a disarmed Ti-plasmid vector.

The selection of the female-sterility DNA of this invention is not critical. A suitable female-sterility DNA can be selected and isolated in a well-known manner, so that it encodes the first RNA, protein or polypeptide which significantly disturbs adversely the proper metabolism and/or functioning and/or development of any cell of a flower and/or seed and/or embryo in which the female-sterility DNA is expressed, preferably leading thereby to the death of such cell. Preferred examples of female-sterility DNAs encode: RNases such as RNase T1 (which degrades RNA molecules by hydrolyzing the bond after any guanine residue) and Barnase; DNases such as an endonuclease (e.g., Eco RI); or proteases such as a papain (e.g., papain zymogen and papain active protein). Other preferred examples of female-sterility DNAs encode: ribonucleases such as T_2 (Kawata et al (1988) Eur. J. Biochem 176 , 683-697) or Rh (Horiuchi et al (1988) J. Biochem 103 , 408-418); or glycoproteins such as are encoded by the S1, S2, S3, S6 and S7 alleles, particularly of Nicotiana alata (McClure et al (1989) Nature 342 , 955-957).

Other examples of female-sterility DNAs encode enzymes which catalyze the synthesis of phytohormones, such as: isopentenyl transferase which is an enzyme that catalyzes the first step in cytokinin biosynthesis and is encoded by gene 4 of Agrobacterium T-DNA; or one or both of the enzymes involved in the synthesis of auxin and encoded by gene 1 and gene 2 of Agrobacterium T-DNA. Yet other examples of female-sterility DNAs encode: glucanases; lipases such as phospholipase A_2 (Verheij et al (1981) Rev. Biochem. Pharmacol. 91 , 92-203); lipid peroxidases; or plant cell wall inhibitors. Still other examples of female-sterility DNAs encode proteins toxic to plants cells, such as a bacterial toxin (e.g., the A-fragment of diphtheria toxin or botulin).

Still another example of a female-sterility DNA is an antisense DNA: i) which encodes a strand of DNA complementary to a strand of DNA that is endogenous to, and naturally transcribed in, the cells of the flower, seed or embryo of the plant of this invention and ii) which is under the control of an endogenous promoter as described, for example, in European patent publication 0,223,399. Such an antisense DNA can be transcribed into an RNA sequence capable of binding to the coding and/or non-coding portion of an RNA, naturally produced in the cell of the flower, seed or embryo, so as to inhibit the translation of the naturally produced RNA. Examples of such an antisense DNA are the antisense DNAs of: the STMG-type genes, such as the STMG07 gene, the STMG08 gene, the STMG4B12 gene and the STMG3C9 gene of Example 2 herein; the KT13 gene (Jofuku and Goldberg (1989) The Plant Cell 1 , 1079-1093); a gene encoding a seed-specific storage protein, such as a 2S albumin (Krebbers et al (1988) Plant Physiol. 87 , 859-866; Altenbach et al (1987) Plant Molecular Biol. 8 , 239-250; (Scolfield and Crouch (1987) J. Biol. Chem. 262 , 12202-12208); or a gene corresponding to cDNA clone pMON9608 (Gasser et al (1989) The Plant Cell 1 , 15). Such antisense DNAs can be naturally expressed in flower, seed or embryo cells of the plant under the control of the endogenous promoter of the complementary endogenous DNA strand (or gene) of the plant, for example: in the style (with the antisense DNA of the STMG07, STMG08, STMG4B12 or STMG3C9 gene); in the embryo axis (with the antisense DNA of the KTI3 gene); in seeds (with the antisense DNA of a 2S albumin-encoding gene); and in ovule cells (with the antisense DNA of PMON9608).

A further example of a female-sterility DNA encodes a specific RNA enzyme (i.e., a so-called "ribozyme"), capable of highly specific cleavage against a given target sequence as described by Haseloff

and Gerlach (1988) Nature 334 , 585-591. Such a ribozyme is, for example, the ribozyme targeted against the RNA encoded by the STMG07 gene, the STMG08 gene, the STMG4B12 gene, the STMG3C9 gene, the KTI3 gene, a gene encoding a seed-specific storage protein such as a 2S albumin or the gene corresponding to cDNA clone pMON9608.

Still other examples of female-sterility DNAs encode products which can render the flowers, seeds and/or embryos susceptible to a specific disease, such as a fungus infection. Such a female-sterility DNA can be used in a plant, in which all other cells or tissues, in which the female-sterility DNA is not expressed, are resistant to the specific disease.

Yet another example of a female-sterility DNA comprises a combination of: 1) a first gene encoding a viral dependent RNA polymerase, such as TNV replicase (Meulewater et al (1990) Virology 177 , 1-11), under the control of a first promoter of this invention; and 2) a negative strand (i.e., antisense DNA) of a second gene: i) which encodes a first protein or polypeptide of this invention that, when produced or overproduced in the plant cell of this invention, disturbs significantly cell metabolism, development and/or functioning, ii) which is fused at its 3′ end to a viral RNA replication recognition sequence or so-called "viral subgenomic promoter", such as the TNV subgenomic promoter (Lexis et al (1990) J. of Virology 64 (4), 1726-1733); and iii) which is fused at its 5′ end to, and under the control of, another suitable promoter, such as a first promoter of this invention, capable of directing expression of the negative strand in the plant cell of this invention. The viral subgenomic promoter sequence is specifically recognized by the viral dependent RNA polymerase encoded by the first gene. This recognition leads to the repeated replication of the negative strand of the second gene as a sense strand, which leads to the synthesis of the first protein or polypeptide. Both the first gene and the negative strand are provided in the nuclear genome of the plant cell of this invention. This can be achieved by one transformation event, by two consecutive transformation events, or by crossing a plant having the first gene inserted into its genome with a plant having the negative strand inserted into its genome.

By "foreign" with regard to the foreign DNA sequence of this invention is meant that the foreign DNA sequence contains a foreign female-sterility DNA and/or a foreign first promoter. By "foreign" with regard to a DNA, such as a female-sterility DNA and a first promoter, as well as a marker DNA, a second promoter and any other DNA in the foreign DNA sequence, is meant that such a DNA is not in the same genomic environment in a plant cell, transformed with such a DNA in accordance with this invention, as is such a DNA when it is naturally found in the cell of the plant, bacteria, animal, fungus, virus, or the like, from which such a DNA originates. This means, for example, that a foreign female-sterility DNA or marker DNA can be: 1) a nuclear DNA in a plant of origin; 2) endogenous to the transformed plant cell (i.e., from a plant of origin with the same genotype as the plant being transformed); and 3) within the same transcriptional unit as its own endogenous promoter and 3′ end transcription regulation signals (from the plant of origin) in the foreign DNA sequence of this invention in the transformed plant cell; but 4) inserted in a different place in the nuclear genome of the transformed plant cell than it was in the plant of origin so that it is not surrounded in the transformed plant cell by the genes which surrounded it naturally in the plant of origin. A foreign female-sterility or marker DNA can also, for example, be: 1) a nuclear DNA in a plant of origin; and 2) endogenous to the transformed plant cell; but 3) in the same transcriptional unit as a different (i.e., not its own) endogenous promoter and/or 3′ end transcription regulation signals in a foreign chimaeric DNA sequence of this invention in a transformed plant cell. A foreign female-sterility or marker DNA can also, for example, be: 1) a nuclear DNA in a plant of origin; and 2) endogenous to the transformed plant cell; but 3) in the same transcriptional unit as a heterologous promoter and/or 3′ end transcription regulation signals in a foreign chimaeric DNA sequence of this invention in a transformed plant cell. A foreign female-sterility or marker DNA can also, for example, be heterologous to the transformed plant cell and in the same transcriptional unit as an endogenous promoter and/or 3′ transcription regulation signals (e.g., from the nuclear genome of a plant with the same genotype as the plant being transformed) in a foreign chimaeric DNA sequence of this invention in a transformed plant cell. An example of a foreign female-sterility DNA could come from the nuclear genome of a plant with the same genotype as the plant being transformed and encode a catalytic enzyme, such as a protease or ribonuclease, that is endogenous to cells of the flowers, seeds and/or embryos of the plant being transformed, so that the enzyme is overproduced in transformed cells of the flowers, seeds and/or embryos in order to disturb significantly their metabolism, functioning and/or development. Preferably, the female-sterility DNA and the marker DNA are each heterologous to the plant cell being transformed.

By "heterologous" with regard to a DNA, such as a female-sterility DNA, a first promoter, a marker DNA, a second promoter and any other DNA in the foreign DNA sequence of this invention, is meant that such a DNA is not naturally found in the nuclear genome of cells of a plant with the same genotype as the plant being transformed. Examples of heterologous DNAs include chloroplast and mitochondrial DNAs

obtained from a plant with the same genotype as the plant being transformed, but preferred examples are chloroplast, mitochondrial, and nuclear DNAs from plants having a different genotype than the plant being transformed, DNAs from animal and bacterial genomes, and chromosomal and plasmidial DNAs from fungal and viral genomes.

By "chimaeric" with regard to the foreign DNA sequence of this invention is meant that at least one of its female-sterility DNAs: 1) is not naturally found under the control of its first promoter for the one female-sterility DNA; and/or 2) is not naturally found in the same genetic locus as at least one of its marker DNAs. Examples of foreign chimaeric DNA sequences of this invention comprise: a female-sterility DNA of bacterial origin under the control of a first promoter of plant origin; and a female-sterility DNA of plant origin under the control of a first promoter of plant origin and in the same genetic locus as a marker DNA of bacterial origin.

By "flower" is meant to include the entire organ of a flower, as well as one or more of its individual parts such as its shoot axis, sepals, petals, male reproductive organs (or stamens) and female reproductive organs (or carpels), whose wholly or partly, retarded or arrested development in accordance with this invention prevents the development of viable seeds in the flower but not the development and propagation of its male gametes; by "female organ" is meant the entire organ of a flower that is involved in the production of female gametes and/or viable seeds and/or viable embryos, as well as one or more of its individual parts such as its ovule, ovary, style, stigma, corolla, disc, septum, calyx and placenta tissue. By "embryo" is meant to include the entire embryo of a plant, as well as one or more of its individual parts such as its embryo axis and embryo cotyledons.

So that the female-sterility DNA of this invention is expressed selectively in cells of the flowers, particularly one or more of their female organs, in cells of the seeds and/or in cells of the embryos of the plants of this invention, it is preferred that the first promoter, which controls the female-sterility DNA in the foreign DNA sequence, be a promoter capable of directing gene expression selectively in cells of the flowers, seeds and/or embryos of the plant. Such a flower-, seed- and/or embryo-specific promoter can be an endogenous promoter or an exogenous promoter and can be from the nuclear genome or from the mitochondrial or chloroplast genome of a plant cell. In any event, the first promoter is foreign to the nuclear genome of the plant cell, being transformed. Preferably, the first promoter causes the female-sterility DNA to be expressed only in cells of one or more specific tissues of the flowers, preferably one or more female organs thereof, or of the seeds or of the embryos, especially in style cells, ovary cells, septum cells, seedcoat cells, endosperm cells, embryo axis cells and/or embryo cotyledon cells.

The first promoter of this invention can be selected and isolated in a well known manner from a plant, to be rendered female-sterile, so that the first promoter directs expression of the female-sterility DNA selectively in cells of the flowers, seeds and/or embryos of the plant, so as to kill or disable the plant's flowers, seeds and/or embryos and render the plant incapable of producing fertile female gametes, viable seeds and/or viable embryos. The first promoter is preferably also selected and isolated so that it is effective to prevent expression of the female-sterility DNA in other parts of the plant that are not involved in the production of fertile female gametes, viable seeds and/or viable embryos, especially in male organs of the flowers, so that the plant remains male-fertile. For example, a suitable flower-specific (preferably female reproductive organ-specific), seed-specific or embryo-specific first promoter can be identified and isolated in a plant, to be made female-sterile, by:

1. searching for an mRNA which is only present in the plant during the development of its flowers, seeds or embryos, preferably its ovary, style, placenta, calyx, scutellum, septum, seedcoat, endosperm or embryo cotyledons;
2. isolating this flower-, seed- or embryo-specific mRNA;
3. preparing a cDNA from this specific mRNA;
4. using this cDNA as a probe to identify the regions in the plant genome which contain DNA coding for this specific mRNA; and then
5. identifying the portion of the plant genome that is upstream (i.e., 5′) from the DNA coding for this specific mRNA and that contains the promoter of this DNA.

Examples of a first promoter of this invention are the Nicotiana tabacum promoters of the STMG-type genes, described in Example 2, which are style and/or stigma specific promoters. Other style-stigma specific first promoters from other plant species can be isolated from their genomes, using the STMG-type genes as a probe as in step 4, above. Under hybridizing conditions, such a probe will hybridize to DNA coding for a style-stigma specific mRNA in a mixture of DNA sequences from the genome of the other plant species (Maniatis et al (1982) Molecular Cloning. A Laboratory Manual. Ed. Cold Spring Harbor Laboratory). Thereafter, as in step 5 above, another style-stigma specific first promoter can be identified. Other style-specific promotors can be isolated from self-incompatibility genes, such as an S-gene, for example as

isolated from Nicotiana alata (McClure et al (1989) Nature 342 , 955-957). Other female organ-specific promoters can be identified using other female organ-specific cDNAs, such as cDNA clone pMON9608 (Gasser et al (1989) The Plant Cell 1 , 15) that hybridizes exclusively with a gene expressed only in the ovules of tomato plants.

Other examples of such a first promoter are: the promoter of the KTI3 gene (Jofuku and Goldberg (1989) The Plant Cell 1 , 1079-1093) which is an embryo axis-specific promoter; and the seed-specific promoters derived from genes encoding seed-specific storage proteins, such as the PAT2S promoters, for example PAT2S1, PAT2S2, PAT2S3 and PAT2S4 which are promoters of the four 2S albumin genes ("AT2S genes") of Aribidopsis thaliana (Krebbers et al (1988) Plant Physiol. 87 , 859-866).

If more than one female-sterility DNA is present in the foreign DNA sequence of this invention, all the female-sterility DNAs can be under the control of a single first promoter, but preferably, each female-sterility DNA is under the control of its own separate first promoter. Where a plurality of female-sterility DNAs are present in the foreign DNA sequence, each female-sterility DNA can encode the same or different first RNA, polypeptide or protein. For example, when the female-sterility DNA encodes an RNase such as RNase T1, it is preferred that at least 3, particularly 4 to 6, copies of the female-sterility DNA and its first promoter be provided in the foreign DNA sequence. In such a case it is also preferred that all the female-sterility DNAs and their first promoters be adjacent to one another in the foreign DNA sequence and in any vector used to transform plant cells with the foreign DNA sequence. If the plurality of female-sterility DNAs encode different products, such as gene 1 and gene 2 or such as TNV replicase and a RNase, DNase or protease, it may be preferred that the female-sterility DNAs not be adjacent to one another and perhaps not even be present in the same vector used to transform plant cells with the foreign DNA sequence or even not present in the same parent plant of the female-sterile plant of this invention.

The selection of the marker DNA of this invention also is not critical. A suitable marker DNA can be selected and isolated in a well known manner, so that it encodes a second RNA, protein or polypeptide that allows plants or their tissue, seeds or even cells, expressing the marker DNA, to be easily distinguished and separated from plants or their tissue, seeds or even cells not expressing the second RNA, protein or polypeptide. Examples of marker DNAs encode proteins that can provide a distinguishable color to plant cells, such as the A1 gene encoding dihydroquercetin-4-reductase (Meyer et al (1987) Nature 330 , 677-678) and the glucoronidase gene (Jefferson et al (1988) Proc. Natl. Acad. Sci. USA ("PNAS") 83 , 8447), or that provide a specific morphological characteristic to the plant such as dwarf growth or a different shape of the leaves. Other examples of marker DNAs confer on plants: stress tolerance, such as is provided by the gene encoding superoxide dismutase as described in European patent application 88/402222.9; disease or pest resistance such as is provided by a gene encoding a Bacillus thuringiensis endotoxin conferring insect resistance as described in European patent application 86/300291.1 or a gene encoding a bacterial peptide that confers a bacterial resistance as described in European patent application 88/401673.4.

Preferred marker DNAs encode second proteins or polypeptides inhibiting or neutralizing the action of herbicides such as: the sfr gene and the sfr v gene encoding enzymes conferring resistance to glutamine synthetase inhibitors such as Biolaphos and phosphinotricine as described in European patent application 87/400,544.0; and genes encoding modified target enzymes for certain herbicides that have a lower affinity for the herbicides than naturally produced endogenous enzymes, such as a modified glutamine synthetase as a target for phosphinotricine as described in European patent publication 0,240,792 and a modified 5-enolpyruvylshikimate-3 phosphate synthase as a target for glyphosate as described in European patent publication 0,218,571. Other examples are: marker DNAs encoding proteins which neutralize the action of the herbicide bromoxynil (Stalker et al (1988) in: Genetic Improvements of Agriculturally Important Crops, Ed: R.T. Fraley, N.M. Frey and J. Schell, Cold Spring Harbor Laboratories); the herbicide sulfonylurea (Lee et al (1988) EMBO J. 7 , 1241-1248); and the herbicide 2,4 D (presented at the 2nd International Symposium of Plant Molecular Biology, Jerusalem, 13-18 November 1988).

The second promoter of this invention, which controls the marker DNA, can also be selected and isolated in a well known manner so that the marker DNA is expressed either selectively in one or more specific tissues or specific cells or constitutively in the entire plant, as desired depending on the nature of the second RNA, protein or polypeptide encoded by the marker DNA. For example, if the marker DNA encodes an herbicide resistance, it may be useful to have the marker DNA expressed in all cells of the plant, using a strong constitutive second promoter such as a 35S promoter (Odell et al (1985) Nature 313 , 810-812), a 35S′3 promoter (Hull and Howell (1987) Virology 86 , 482-493), the promoter of the nopaline synthetase gene ("PNOS") of the Ti-plasmid (Herrera-Estrella (1983) Nature 303 , 209-213) or the promoter of the octopine synthase gene ("POCS" [De Greve et al (1982) J. Mol. Appl. Genet. 1 (6), 499-511]). If the marker DNA encodes a protein conferring disease resistance, it may be useful to have the marker DNA selectively expressed in wound tissue by using, for example, a TR promoter such as the TR1′ or TR2′

promoter of the Ti-plasmid (Velten et al (1984) EMBO J. 3 , 2723-2730). If the marker DNA encodes a herbicide resistance, it also may be useful to have the marker DNA selectively expressed in green tissue by using, for example, the promoter of the gene encoding the small subunit of Rubisco (European patent application 87/400,544.0). If the marker DNA encodes a pigment, it also may be useful to have the marker DNA expressed in specific cells such as petal cells, leaf cells or seed cells, preferably in the outside layer of the seed coat.

One can identify and isolate in a well known manner a tissue-specific second promoter for a plant to be rendered female-sterile and easily distinguishable from non-transformed plants by:

1. searching for an mRNA which is only present in the plant during the development of a certain tissue, such as its petals, leaves or seeds;
2. isolating this tissue-specific mRNA;
3. preparing a cDNA from this tissue-specific mRNA;
4. using this cDNA as a probe to identify the regions in the plant genome which contain DNA coding for the tissue-specific mRNA; and then
5. identifying the portion of the plant genome that is upstream from the DNA coding for the tissue-specific mRNA and that contains the promoter for said DNA.

If more than one marker DNA is present in the foreign DNA sequence of this invention, all the marker DNAs can be under the control of a single second promoter, but preferably, each marker DNA is under the control of its own separate second promoter. More preferably, each marker DNA is under the control of its own second promoter and encodes a different second RNA, protein or polypeptide, providing different distinguishable characteristics to a transformed plant. In some cases, it may be preferred that the marker DNA(s) and second promoter(s) are adjacent to each other and to the one or more female-sterility DNAs contained in the foreign DNA sequence of this invention and in any vector used to transform plant cells with the foreign DNA sequence. In other cases, it may be preferred that the marker DNAs are not adjacent to each other and/or to the female-sterility DNAs.

It is generally preferred that the first RNA, protein or polypeptide, encoded by the female-sterility DNA, interfere significantly with the metabolism, functioning and/or development of the cells of the flowers and/or seeds and/or embryos by acting in the cytoplasm or the nucleus of these cells. However, when it is desired to have the first protein or polypeptide and/or the second protein or polypeptide transported from the cytoplasm into chloroplasts or mitochondria of the cells of transformed plants, the foreign DNA sequence can further include a first additional foreign DNA encoding a transit peptide. The first additional DNA is located between the female-sterility DNA and the first promoter if the first protein or polypeptide is to be so-transported and is between the marker DNA and the second promoter if the second protein or polypeptide is to be so-transported. By "transit peptide" is meant a polypeptide fragment which is normally associated with a chloroplast or mitochondrial protein or subunit of the protein that is produced in a cell as a precursor protein encoded by the nuclear DNA of the cell. The transit peptide is responsible for the translocation process of the nuclear-encoded chloroplast or mitochondrial protein or subunit into the chloroplast or the mitochondria, and during such a process, the transit peptide is separated or proteolytically removed from the chloroplast or mitochondrial protein or subunit. One or more of such first additional DNAs can be provided in the foreign DNA sequence of this invention for transporting one or more first or second proteins or polypeptides as generally described in European patent applications 85/402,596.2 and 88/402,222.9 and in: Van den Broeck et al (1985) Nature 313 , 358-363; Schatz (1987) Eur. J. of Bioch. 165 , 1-6; and Boutry et al (1987) Nature 328 , 340-342. An example of a suitable transit peptide for transport into chloroplasts is the transit peptide of the small subunit of the enzyme RUBP carboxylase (European patent application 85/402,596.2), and an example of a transit peptide for transport into mitochondria is the transit peptide of the enzyme Mn-superoxide dismutase (see example 10 herein and European patent application 89/401,194.9).

It is also generally preferred that the first RNA, protein or polypeptide, encoded by the female-sterility DNA, act intracellularly so as to interfere with cell metabolism, functioning and/or development in the plant. However when it is desired to have the first protein or polypeptide and/or the second protein or polypeptide secreted out of the intercellular areas of the plant cells, in which they are expressed, or out of the tissue, in which they are expressed, the foreign DNA sequence can further include a second additional foreign DNA encoding a secretory signal peptide. The second additional foreign DNA is located between the female-sterility DNA and the first promoter if the first protein or polypeptide is to be secreted and between the marker DNA and the second promoter if the second protein or polypeptide is to be secreted. By "secretory signal peptide" is meant a natural polypeptide fragment which is, particularly in eukaryotic cells, associated during translocation with proteins that are normally secreted from c lls or an artificial polypeptide fragment which, when associated during translocation with a protein or polypeptide, provokes its secretion from cells.

Examples of suitable secretory signal peptides are set forth in: Von Heijne (1986), NAR 14 (11), 4683-4690; Denecke et al (1990), The Plant Cell 2 , 51-59; and Chrispeels and Taque (1990) International Review of Cytology, in press.

In the foreign DNA sequence of this invention, 3′ transcription termination and polyadenylation signals can be selected in a conventional manner from among those which are capable of providing correct transcription termination and polyadenylation of mRNA in plant cells. The transcription termination and polyadenylation signals can be the natural ones of the gene to be transcribed but can also be foreign or heterologous. Examples of heterologous transcription termination and polyadenylation signals are those of the octopine synthase gene (Gielen et al (1984) EMBO J. 3 , 835-845) and the T-DNA gene 7 (Velten and Schell (1985) Nucleic Acids Research ("NAR") 13 , 6981-6998).

Also in accordance with this invention, plant cell cultures, containing the foreign DNA sequence of this invention, can be used to regenerate homozygous dominant female-sterile male-fertile plants by performing the necessary transformations on diploid (Chuong and Beversdorf (1985) Plant Sci. 39 , 219-226) or on haploid cell cultures and then (for haploid cell cultures) doubling the number of chromosomes by well known techniques (e.g., with colchicine). See: Plant Tissue and Cell Culture, Plant Biology 3 , A.R. Liss, Inc. N.Y. (1987). Thereby, the foreign DNA sequence will be in homozygous form in the nuclear genome of each of the so-transformed plant cells. This is preferred for plant cell cultures containing a female-sterility DNA under the control of a first promoter which directs gene expression at a given stage of development of the female gametes, such as ovules, especially after meiosis, or in cells derived from the female gametes, such as seed or embryo cells, so that the female-sterility DNA is present and can be expressed in all female gametes or plant cells derived therefrom.

Also in accordance with this invention, processes are provided for producing hybrid seeds which can be grown into hybrid plants. These processes involve crossing in an otherwise conventional manner: a) a nuclear female-sterile male-fertile plant of this invention, in which the first RNA, protein or polypeptide is expressed selectively in flowers, preferably in at least one female organ thereof, or in embryos; with b) a male-sterile female-fertile plant. Suitable male-sterile female-fertile plants are described in European Patent Application 89/401194.9 as having a nuclear genome, in which are stably integrated:

(a) a male-sterility DNA encoding a RNA, protein or polypeptide which, when produced or overproduced in a stamen cell of a plant, significantly disturbs adversely the metabolism, functioning and/or development of the stamen cell; and

(b) a promoter capable of directing expression of the male-sterility DNA selectively in stamen cells of the plant, preferably in anther, pollen and/or filament cells, particularly in tapetum and/or anther epidermal cells; the male-sterility DNA being in the same transcriptional unit as, and under the control of, this promoter; and which optionally has in the same genetic locus:

(c) another marker DNA encoding a RNA, protein or polypeptide which, when present at least in a specific tissue or in at least specific cells of the plant, renders the plant easily separable from other plants which do not contain this RNA, protein or polypeptide at least in the specific tissue or specific cells; and

(d) another promoter capable of directing expression of the other marker DNA at least in the specific tissue or specific cells and being in the same transcriptional unit as, and controlling, the other marker DNA. The female-sterile plants and male-sterile plants are planted at random, near to each other to increase the chances of cross-pollination, without the need for precise planting patterns. The harvested seed, which is capable of germinating, will be the result of the fertilization of the male-sterile plants by the female-sterile plants and will be 100% hybrid. When the foreign DNA sequences responsible for the female-sterility and male-sterility characteristics are present in heterozygous form in the nuclear genomes of the respective parent plants, plants grown from such hybrid seed will be: 25 % fertile, 25 % female-sterile, 25 % male-sterile and 25 % sterile. When the foreign DNA sequence encoding female-sterility is present in the nuclear genome of the male-fertile parent plant in homozygous form--which is preferred when the first promoter is an ovule-, seed- or embryo-specific promoter--all the plants grown from such hybrid seed will be female-sterile.

Further in accordance with this invention, processes are provided for producing fruit without seeds by crossing in an otherwise conventional manner: a) a nuclear female-sterile male-fertile plant of this invention, in which the first RNA, protein or polypeptide is expressed selectively in seeds and in which the foreign DNA sequence, encoding the first RNA, protein or polypeptide, is preferably in homozygous form in the nuclear genome of the plant; with b) a male-sterile female-fertile plant.

Plants, transformed with the female-sterility DNA and in some cases preferably also with the marker DNA encoding an herbicide resistance, stably integrated in the plants' nuclear genomes and transmissible throughout generations as dominant alleles in accordance with this invention, are alternatives to, and can

provide advantages over, presently used cytoplasmic and nuclear male-sterility systems for breeding and producing hybrid crops. In this regard, female-sterile male-fertile plants can provide: 1) inhibited seed formation in crops, 2) hybrid seeds for crops which do not easily cross-pollinate, and 3) easier breeding of plant lines as discussed below.

1. Inhibition of seed formation

There exist a wide variety of crops cultivated by man in which the seed is an undesirable by-product.

a) When the economic product of a plant consists of its vegetative part. By inhibiting seed production, the plant's energy can be focused on vegetative biomass production. Examples are perennial plants (e.g. forage grasses, forage legumes and rubber trees), some annual plants (e.g., sugar cane and potato), and especially all crops that would normally flower and set seed before the economic product is harvested. Other examples are plants, obtainable through genetic engineering, which produce, within their vegetative tissues, proteins, polypeptides or other metabolites for pharmaceutical or industrial purposes.

b) When the economic product of a plant is its fruit and it is desirable that the fruit be seedless, either because of consumer preferences (e.g., in tomato, melon and citrus fruit) or because seed formation uses up biomass that could otherwise be stored in the fruit (e.g., for providing high solids in tomatoes to be processed). Since such crops require fruit formation, the seedless condition, which usually induces fruit abortion, has to be compensated for by the possibility of obtaining parthenocarpic fruit set. Natural parthenocarpic fruit inducing genes exist in some crops, such as tomato and melon.

c) When the plant is not grown for its seeds, but remaining propagules after harvesting may give rise to seed formation. The regrowth from these seeds can cause a considerable weed problem in the next culture. This "weed" problem is particularly well known with sugar beet.

d) When the plant is grown for its flowers (e.g., cut flowers, pot plants or garden ornamentals). For these species, it is often desirable to avoid seed set. In the case of cut flowers or pot plants, fertilization of the flowers often induces an accelerated senescence of the petals. In case of garden ornamentals, the formation of fruits and seeds reduces the time span and the intensity of flowering.

2. Hybrid seed production

Engineered female-sterility is useful as a seed production tool in combination with natural cytoplasmic or nuclear male-sterility systems or engineered nuclear male-sterility systems for the production of commercial hybrid seeds in crops where the seed is not the economic harvest and which do not easily cross- pollinate (e.g., for forage grasses, forage legumes, sugar beet, and many vegetables). The breeding of nuclear female-sterile plants with male-sterile plants provides a better control of hybrid seed quality (e.g., no mistakenly harvested male rows) and a higher seed set by favoring cross-pollination through at random interplanting of male-sterile and female-sterile parent plants and does not need the use of a restorer of fertility. A strategy for such production of hybrid seeds (e.g., for sugar-beet) may include the following steps ("MS" stands for male-sterility, "FS" stands for female-sterility and "H" stands for herbicide resistance):

A. Development of the female parent line A

Aa) Transform line A with a foreign DNA including a male-sterility DNA under the control of a stamen specific promoter and adjacent thereto a marker DNA encoding herbicide resistance, according to European patent application 89/401,194.9, giving $A^{MSH/msh}$

Ab) Maintain line $A^{MSH/msh}$ through crossing with line $A^{msh/msh}$. This gives:

50% $A^{MSH/msh}$ (male-sterile, herbicide resistant) and

50% $A^{msh/msh}$ (fertile, herbicide sensitive).

B. Development of the male parent line B

Ba) Transform line B with the chimaeric DNA sequence of this invention including a female-sterility DNA under the control of a first promoter which directs gene expression selectively in cells of a female organ of the plant and adjacent thereto a marker DNA encoding herbicide resistance, giving $B^{FSH/fsh}$.

Bb) Maintain line $B^{FSH/fsh}$ through crossing with $B^{fsh/fsh}$, yielding:

50% $B^{FSH/fsh}$ (female-sterile,herbicide resist.) and
50% $B^{fst/fsh}$ (fertile, herbicide sensitive).

C. Producing the hybrid seed crop

Ca) Planting seeds obtained in Ab) and Bb) at random.
Cb) Eliminating through spraying with the herbicide the undesirable genotype before cross- and self-pollination could occur.
Cc) Cross-pollination occurring:
$A^{MSH/msh} \times B^{FSH/fsh}$
giving 100% hybrid seeds with the following genotype:
25% $AB^{MSH/msh;\ FSH/fsh}$
25% $AB^{MSH/msh;\ fsh/fsh}$
25% $AB^{msh/msh;\ FSH/fsh}$
25% $AB^{msh/msh;\ fsh/fsh}$
This represents the commercially sold seed.

3. Easier Breeding

a) without a marker DNA

The ability to obtain microspore-derived double haploids of most major crops allows the production of homozygous nuclear female-sterile lines in a more or less straightforward way (Chuong and Beversdorf (1985) Plant Sci. 39 , 219-226). This makes it unnecessary in many cases to have a marker DNA within the same genetic locus of the nuclear genome of the cells of the plant as the female-sterility DNA. This is especially so if the homozygous female-sterile plant can be vegetatively multiplied (e.g., many vegetables).

b) with a marker DNA

In case the female-sterility DNA is in the same genetic locus of the nuclear genome of the transformed plant as a marker DNA (e.g., encoding herbicide resistance), homozygous female-sterile plants are technically superior to many other lines as tester parents in line evaluation programs. This is especially the case for crops where the seed is not the economic harvest and which can easily cross-pollinate. Indeed, these female-sterile plants allow the testing of many female- and male-fertile lines in close proximity to one another while making it easy to eliminate any self-pollinated seed from the different plant lines, being tested, from seeds resulting from crosses between the different lines.

The following Examples illustrate the invention. The figures referred to in the Examples are as follows:

Fig. 1A shows the cDNA sequence of the STMG07 gene of Example 1.
Fig. 1B shows the cDNA sequence of the STMG08 gene of Example 1.
Fig. 2A shows the cDNA sequence of the STMG4B12 gene of Example 1.
Fig. 2B shows the cDNA sequence of the STMG3C9 gene of Example 1.
Fig. 3 shows a map of the vector pMG100 of Example 4.
Fig. 4 shows a map of the vector pMG101 of Example 6.
Fig. 5 shows a map of the vector pMG102 of Example 8.
Fig. 6 shows a map of the vector pMG103 of Example 8.
Fig. 7 shows a map of the vector pMG104 of Example 10.
Fig. 8 shows a map of the vector pMG105 of Example 10.

Unless otherwise stated in the Examples, all procedures for making and manipulating recombinant DNA were carried out by the standardized procedures described in Maniatis et al, Molecular Cloning - A Laboratory Manual , Cold Spring Harbor Laboratory (1982). The following vector, used in the Examples, has been deposited in the Deutsche Sammlung Für Mikroorganismen und Zellculturen ("DSM"), Mascheroder Weg 1B, D-3300 Braunschweig, Federal Republic of Germany under the provisions of the Budapest Treaty:

Vector	DSM Accession No.	Date
pGSC1700	4469	21 Mar. 1988

Example 1 - Isolation of style-stigma specific cDNAs from Nicotiana tabacum "Petit Havana" SR1 .

Using well known procedures (Maniatis et al, 1982), total mRNA was isolated from the following different tobacco tissues: style-stigma tissues from flowers in stage 3 to 7 (according to Goldberg (1988) Science 240 , 1460-1467); so-called "young stage" style-stigma tissues from a flower which did not develop pollen grains in stage 8 to 11 (according to Goldberg, 1988); so-called "old stage" ovary tissue from flowers in young stage; ovary tissue from flowers in old stage; and stem, root and leaf tissue from in vitro cultivated seedlings. cDNAs were synthesized from young and old style-stigma tissues using the Amersham (Amersham International PLC, Buckinghamshire, England) kit, cDNA Synthesis System Plus-RPN 1256 Y/Z, according to the directions set forth in the kit for its use. The cDNAs were cloned in Lambda gt 10 vector using the Amersham kit, cDNA Cloning System-lambda gt 10 - RPN 1257, according to the directions set forth in the kit. From the cDNA library thus obtained, differential screening was performed with a cDNA probe from seedlings on the one hand and a cDNA probe from style-stigma tissues on the other hand. The selected clones were subcloned in pGEM1 (Promega, Madison, Wisconsin, USA). Probes of each of these subclones were prepared and first checked for their specificity in Northern blots with 10 ug of total mRNA from different tobacco tissues (roots, stems, leaves, sepals, petals, anthers, young stage style-stigma, old stage style-stigma, and old stage ovaries). The subclones, that specifically hybridized in these Northern blots with style-stigma mRNA, were again hybridized in Northern blots with 2 ug poly A^+ mRNA isolated from the above-mentioned tissues, including young ovaries, seeds and virus-infected leaves. The clones, called "pMG07" and "pMG08" and containing an insert of 0.963 kb and 0.472 kb, respectively, proved to be style-stigma specific cDNA sequences. These clones were sequenced, and their cDNA sequences are shown in Fig. 1A and Fig. 1B, respectively. The DNA sequence of pMG07 reveals the presence of one open reading frame ("ORF") over a sequence of 800 nucleotides. The sequence of pMG08 reveals an ORF over the total sequence.

From Prof. Goldberg of the University of California, Los Angeles (UCLA) were obtained: two Nicotiana tabacum style-stigma specific cDNAs (4B12 and 3C9) cloned as a Pst I - Sma I fragment in pGEM 3zf (-) (Promega, Madison, Wisconsin, USA). These clones contained inserts of 0.748 kb and 1.046 kb, respectively. Probes of these two clones were hybridized in Northern blots with 10 ug of total mRNA from different tobacco tissues (roots, stems, leaves, sepals, petals, anthers, young stage style-stigma, old stage style-stigma, and old stage ovaries) in order to check their specificity. These Northern blots confirmed the specificity of the clones and revealed that the transcript of 4B12 is 0.8 kb and that of 3C9 is 1.2 kb. The two clones were subcloned in pGEM1 (Promega), which subclones were called "pMG4B12" for the 4B12 clone and "pMG3C9" for the 3C9 clone. The subclones were again checked for their specificity in Northern blots with 2 ug poly A^+ mRNA isolated from the above-mentioned tissues, including young ovaries, seeds and virus- infected leaves. The clones pMG4B12 and pMG3C9, containing inserts of 0.748 kb and 1.046 kb, respectively, proved to be style-stigma specific sequences. These clones were sequenced and their cDNA sequences are shown in Fig. 2A and Fig. 2B, respectively.

Example 2 - Isolation of the style-stigma specific genes ("STMG-type genes") corresponding to the style-stigma cDNA clones

Using known procedures (Maniatis et al, 1982), a probe from each of the cDNA clones of Example 1 of style-stigma specific sequences was used to isolate the corresponding genomic gene sequence which is specifically expressed in style-stigma tissues of the female organ of tobacco. According to protocols provided by Promega, tobacco genomic DNA was partially digested with Sau 3A, and the restriction fragments were cloned into the lambda phage vector GEM.12 (Promega), digested with Xho I to produce genomic clones called "lambda STG07", "lambda STG08", "lambda STG4B12" and "lambda STG3C9". Subsequently, these genomic clones were subcloned in pGEM1 (Promega) according to the procedure of

Promega. The subclones were again analyzed by Southern blot, using the respective cDNA clones as probes in order to identify the clones which contained the style-stigma specific DNA sequences. These subclones, called respectively "pSTG07", "pSTG08", "pSTG4B12" and "pSTG3C9" were sequenced (Maxam and Gilb rt (1977) PNAS 74 , 560). The orientation of these clones was determined by Northern blot analysis with riboprobes of both senses. Comparison of each cDNA sequence with its respective genomic clone sequence led to the identification of the region of homology. At the 5′ end of each region, the ATG codon and the consensus sequence TATA were determined. That the "TATA" box is part of the promoter of the gene is confirmed by primer extention (Mcknight et al (1987) Cell 25 , 385). The style-stigma specific genes, isolated using the style-stigma cDNA as probe, are called in general "STMG-type" genes. The style-stigma specific gene of pSTG07 is called "STMG07", that of pSTG08 is called "STMG08", that of pSTG4B12 is called "STMG4B12" and that of pSTG3C9 is called "STMG3C9".

Example 3 - Construction of promoter cassettes ("PSTMG") derived from the respective STMG-type genes

To construct chimaeric DNA sequences containing 5′ regulator sequences, including the promoter of an STMG-type gene, in the same transcriptional unit as, and controlling, a first heterologous female-sterility DNA, cassettes are constructed by subcloning a DNA fragment including a promoter into the polylinker of pMAC 5-8 (European patent application 87/402348.4). This produces respective vectors which can be used to isolate single strand DNA for use in site directed mutagenesis.

Using site directed mutagenesis (European patent application 87/402348.4), the sequence surrounding the ATG initiation codon of each of the genes is modified in such a way that the mutation creates a given sequence which is a unique recognition site for a given restriction enzyme. The resulting plasmids each contain the newly created restriction site. The precise nucleotide sequence spanning the newly created restriction site is determined in order to confirm that it only differs from the 5′ sequence of the corresponding STMG-type gene by the substitution, creating the new restriction site. The newly created promoter cassettes, each comprising a promoter, a 5′ untranslated end of an STMG-type gene to its ATG initiation codon, and a new restriction site, are generally called "PSTMGs". The PSTMG containing the promoter and 5′ end of STMG07 is called "PSTMG07", that of STMG08 is called "PSTMG08", that of STMG4B12 is called "PSTMG4B12" and that of STMG3C9 is called "PSTMG3C9".

Example 4 - Construction of chimaeric DNA sequences of a PSTMG and a RNAse T1 gene

Plasmids named "pMG100", shown in Fig. 3, are constructed, each by assembling the following well known DNA fragments with a different one of the PSTMGs of Example 3:

1. a vector fragment, including T-DNA border sequences, from pGSC1700 in which the β-lactamase gene encoding ampicillin has been inactivated by insertion into the Sac I site; located between the border sequences are the following DNA fragments 2-4;
2. a chimaeric sequence containing an Arabidopsis Rubisco SSU promoter ("PSSU" or "PSSUARA"), a herbicide resistance gene sfr (European patent application 87/400,544.0) and the 3′ end (i.e., transcription termination) signals of a T-DNA gene 7 (Velten and Schell (1985) NAR 13 , 6981);
3. a chimaeric sequence containing the Eco RI/ Sac I fragment from pGSFR401 which contains a nopaline-synthase promoter ("PNOS"), a neo gene encoding kanamycin resistance and the 3′ end signals of an octopine synthase ("OCS") gene (European patent application 87/400,544.0, wherein pGSFR401 is called "pGSR4"); and
4. a chimaeric sequence, containing one of the PSTMG promoter cassettes from Example 3, fused in frame with a synthetic gene encoding RNase T1 from A. orhyzae , (Quaas et al, "Biophosphates and their Analogues-Synthese, Structure, Metabolism and Activity" (1987) Elsevier Science Publisher B.V., Amsterdam; Quaas et al (1988) Eur. J. Biochem. 173 , 617-622) and the 3′ end signals of a nopaline synthase ("NOS") gene (An et al (1985) EMBO J. 4 (2), 277).

Each pMG100 is a binary type T-DNA vector containing, within the T-DNA border sequences, three chimaeric sequences: PSSU-sfr and PNOS-neo which are marker DNAs with respectively PSSU and PNOS as second promoters; and PSTMG-RNase T1 gene which is a female-sterility DNA under the control of a PSTMG as a first promoter. Expression of the female-sterility DNA under the control of the PSTMG promoter will produce RNase T1 selectively in style and/or stigma cells. This will be lethal for the style and/or stigma cells since the RNase T1 will degrade the RNA molecules which are indispensable for these

cells' metabolism.

Example 5 - Introduction of each chimaeric DNA sequence of Example 4 into tobacco and alfalfa

A recombinant Agrobacterium strain is constructed by mobilization of each pMG100 (from Example 4) from E. coli into Agrobacterium tumefaciens C58C1 Rif^R containing pMP90 (Koncz and Schell (1986) Mol. Gen. Genetics 204 , 383-396).

The resulting Agrobacterium strain, harboring pMP90 and pMG100, is used for the transformation of tobacco leaf discs (N. tabacum Petite Havane SR1), using standard procedures as described, for example, in European patent application 87/400,544.0, and of alfalfa according to the procedure described in D'Halluin et al (1990) Crop Science 30 , in press. Carbenicillin is used to kill the Agrobacterium strains after co-cultivation. Transformed calli are selected on a substrate containing 5 mg/l phosphinotricin and 100 ug/ml kanamycin, and resistant calli are regenerated into plants. After induction of shoots and roots, which proves normal growth of the plants despite the presence of the RNase T1 gene, the transformants are transferred to the greenhouse and are grown until they flower. The flowers are examined and show no normal style-stigma formation. After pollination, no viable seeds are formed. The transformed plants are female-sterile.

Example 6 - Construction of chimaeric DNA sequences of a PSTMG and a Barnase gene

Plasmids named "pmg101", shown in Fig.4, are constructed, each by assembling the following well known DNA fragments with a different one of the PSTMGs of Example 3:

1. a vector fragment, including T-DNA border sequences, derived from pGSC1700 as described in Example 4 and with the following DNA fragments 2-4 between its border sequences;
2. the chimaeric sequence (no. 2) of Example 4, containing the PSSU promoter, the herbicide-resistance gene sfr and the 3′ end of T-DNA gene 7;
3. the chimaeric sequence (no. 3) of Example 4, containing the PNOS promoter, the neo gene encoding kanamycin resistance and the 3′ end signals of the OCS gene; and
4. a chimaeric sequence, containing one of the PSTMGs from Example 3, fused in frame with the Barnase gene from Bacillus amiloliquefaciens (Hartley and Rogerson (1972) Preparative Biochemistry 2 (3), 243-250) and the 3′ end of the NOS gene of Example 4.

Each pMG101 is a binary type T-DNA vector containing, within the T-DNA border sequences, three chimaeric sequences: PSSU-sfr and PNOS-neo which are markers DNAs with respectively PSSU and PNOS as second promoters; and PSTMG-Barnase gene which is a female-sterility DNA under the control of a PSTMG as a first promoter. Expression of the female-sterility DNA under the control of the PSTMG promoter will produce Barnase selectively in style and/or stigma cells. This will be lethal for the style and/or stigma cells since Barnase will degrade the RNA molecules and thereby interfere with the metabolism of these cells.

Example 7 - Introduction of each chimaeric DNA sequence of Example 6 into tobacco and alfalfa

As described in Example 5, a recombinant Agrobacterium strain is constructed by mobilizing each pMG101 (from Example 6) from E. coli into Agrobacterium C58C1 Rif^R containing pMP90 (Koncz and Schell (1986) Mol. Gen. Genetics 204 , 383-396). The resulting strain, harboring pMP90 and pMG101, is used for tobacco leaf disc transformation and for alfalfa transformation. Transformed calli and shoots are selected using 5mg/l phosphinothricin and 100 ug/ml kanamycin. That the Barnase gene is not expressed in the transformed herbicide-resistant calli and shoots is shown by their growth.

The transformed shoots are rooted, transferred to soil in the greenhouse and grown until they flower. The flowers of both the tobacco and alfalfa are examined, and essentially the same phenotype is observed in the transformed plants as is observed in the transformed plants described in Example 5 (i.e., no normal style- stigma formation). The transformed plants are female-sterile.

Example 8 - Construction of chimaeric DNA sequences of a PSTMG and a gene encoding papain

Plasmids named "pMG102", shown in Fig 5, are constructed, each by assembling the following well known DNA fragments with a different one of the PSTMGs of Example 3:

1. a vector fragment, including T-DNA border sequences, derived from pGSC1700 as described in Example 4 and with the following DNA fragments 2-4 between its border sequences;
2. the chimaeric sequence (no. 2) of Example 4, containing the PSSU promoter, the herbicide resistance gene sfr and the 3′ end of T-DNA gene 7.
3. the chimaeric sequence (no. 3) of Example 4, containing the PNOS promoter, the neo gene and the 3′ end of the OCS gene; and
4. a chimaeric sequence, containing one of the PSTMGs from Example 3, fused in frame with:
   a) a papain gene from Carica papaya fruit, encoding the papain zymogen which is a plant endopeptidase (Cohen et al (1986) Gene 48 , 219-227) capable of attacking peptide, as well as ester, bonds; the following modifications are made in the DNA sequence of the papain gene according to Cohen et al (1986), using site directed mutagenesis as described in Example 3:
      i. the nucleotide A, position-1 upstream of the first ATG codon, is mutated into nucleotide C in order to obtain a suitable Nco I cloning site; and
      ii. the GAA codons encoding glutamate at positions 47, 118 and 135 are mutated into CAA codons encoding glutamine; and
   b) the 3′ end of the NOS gene of Example 4.

Each pMG102 is a binary type T-DNA vector containing, within the T-DNA border sequences, three chimaeric sequences: PSSU-sfr and PNOS-neo which are marker DNAs encoding dominant selectable markers for plant transformation under the control of respectively PSSU and PNOS as second promoters; and PSTMG-Papain zymogen gene which is a female-sterility DNA under the control of a PSTMG as a first promoter. Expression of the female-sterility DNA under the control of the PSTMG promoter will produce, selectively in style and/or stigma cells, an endopeptidase (the papain zymogen) that will cleave proteins in the style and/or stigma cells, thus leading to the death of these cells.

Plasmids named "pMG103", shown in Fig.6, are also constructed, each by assembling the following well known DNA fragments with a different one of the PSTMGs of Example 3:

1. a vector fragment, including T-DNA border sequences, derived from pGSC1700 as described in Example 4 and with the following DNA fragments 2-4 between its border sequences;
2. the chimaeric sequence (no. 2) of Example 4, containing the PSSU promoter, the herbicide resistance gene sfr and the 3′ end of T-DNA gene 7;
3. the chimaeric sequence (no. 3) of Example 4, containing the PNOS promoter, the neo gene, and the 3′ end of the OCS gene; and
4. a chimaeric sequence, containing one of the PSTMGs of Example 3, fused in frame with:
   a) a papain gene from Carica papaya fruit, encoding the active protein of the papain zymogen; the following modifications are made in the DNA sequence of the papain gene according to Cohen et al (1986), using site directed mutagenesis as described in Example 3:
      i. the AAT codon encoding Asn, upstream of the first Ile residue of the active protein, is mutated into a GAT codon, which provides a suitable Eco RV cloning site (GAT ATC). The Eco RV engineered site is fused directly to the PSTMG in order to obtain a direct in frame fusion of the promoter with the sequence encoding the active protein of the papain zymogen; and
      ii. the GAA codons encoding glutamate at positions 47, 118 and 135 are mutated into CAA codons encoding glutamine; and b) the 3′ end of the NOS gene of Example 4.

Each pMG103, like each pMG102, is a binary type T-DNA vector containing, within the T-DNA border sequences, three chimaeric genes: PSSU-sfr and PNOS-neo encoding dominant selectable markers for plant transformation; and PSTMG-Papain active protein gene which is a female-sterility DNA that is under the control of a PSTMG as a first promoter and that encodes an endopeptidase that will cleave proteins in style and/or stigma cells, thus leading selectively to the death of these cells.

Example 9 - Introduction of each chimaeric DNA sequence of Example 8 into tobacco and alfalfa

As described in Example 5, each pMG102 and pMG103 (from Example 8) is mobilized from E. coli into separate Agrobacteria C58C1 Rif^R carrying pMP90. The resulting strains, harboring pMP90 with pMG102 and pMP90 with pMG103, are used to transform tobacco and alfalfa following the procedures of Example 5. That the papain genes are not expressed in transformed herbicide- and kanamycin-resistant calli, shoots and roots is shown by their growth.

The transformed plants are transferred into the greenhouse and grown in soil until they flower. The

flowers of both the tobacco and alfalfa are examined, and essentially the same phenotypes are observed in the transformed plants as are observed in the transformed plants described in Example 5 (i.e., no normal style-stigma formation). The transformed plants are female-sterile.

Example 10 - Construction of chimaeric DNA sequences of a PSTMG and a gene encoding EcoRI

Plasmids named "pMG104", shown in Fig. 7, are constructed, each by assembling the following well known DNA fragments with a different one of the PSTMGs of Example 3:

1. a vector fragment, including T-DNA border sequences, derived from pGSC1701A2 (European patent application 87/115985.1); located between the border sequences are the following DNA fragments 2-5;
2. the chimaeric sequence (no. 2) of Example 4, containing the PSSU promoter, the herbicide-resistance gene sfr and the 3′ end of T-DNA gene 7;
3. the chimaeric sequence (no. 3) of Example 4, containing the PNOS promoter, the neo gene and the 3′ end of the OCS gene;
4. a chimaeric sequence, containing one of the PSTMGs of Example 3, fused in frame with:
   a) a gene encoding the Eco RI restriction endonuclease from an *E. coli* (Green et al (1981) J. Biol. Chem. 256 , 2143-2153; Botterman and Zabeau (1985) Gene 37 , 229-239) and capable of recognizing and cleaving the target sequence GAATTC on a double stranded DNA; the following modifications are made in the DNA sequence of the gene according to Green et al (1981) using site directed mutagenesis as described in Example 3:
      i. the nucleotides of the ATG initiation codon are replaced by ATGCA, creating a Nsi I site at the initiation codon and yielding the following nucleotide sequences: ATG CA,TCT,AAT...; and
      ii. the Hind II-Hind III fragment of the Eco RI gene cloned in pEcoR12 (Botterman and Zabeau, 1985) is cloned into the pMAC5-8 site directed mutagenesis vector; and
   b) the 3′ end of the NOS gene of Example 4; and
5. a gene encoding an Eco RI methylase, under the control of its natural promoter (Botterman and Zabeau, 1985), which is capable of inhibiting the activity of Eco RI in *E. coli* or *Agrobacterium* , in order to overcome potential leaky expression of the Eco RI gene in microorganisms.

Each pMG104 is a binary type T-DNA vector containing, within the T-DNA border sequences, three chimaeric sequences: PSSU-sfr and PNOS-neo which are marker DNAs under the control of respectively PSSU and PNOS as second promoters; and PSTMG-EcoRI endonuclease gene which is a female-sterility DNA under the control of a PSTMG as a first promoter. Expression of the female-sterility DNA under the control of the PSTMG promoter selectively in style and/or stigma cells will produce the Eco RI restriction endonuclease which will cleave double stranded DNA at GAATTC sites (see for review of type II restriction modification systems: Wilson (1988) TIG 4 (11), 314-318) in the style and/or stigma cells, thus leading to the death of these cells.

Plasmids named "pMG105" are also constructed, each by assembling the following well known DNA fragments with a different one of the PSTMGs of Example 3:

1. a vector fragment, including T-DNA border sequences, derived from pGSC1701A2; located between the border sequences are the following DNA fragments 2-5;
2. the chimaeric sequence (no. 2) of Example 4, containing the PSSU promoter, the herbicide-resistance gene sfr and the 3′ end of T-DNA gene 7;
3. the chimaeric sequence (no. 3) of Example 4, containing the PNOS promoter, the neo gene and the neo 3′ end of the OCS gene;
4. a chimaeric sequence, containing one of the PSTMGs of Example 3, fused in frame with:
   a) a gene fragment encoding the transit peptide of the Mn-superoxide dismutase ("Mn-SOD") which is a Nco I-Pst I fragment of a Hpa I-Hind III fragment from pSOD1 (Bowler et al (1989) Embo J. 8 , 31-38); the following modifications are made in the DNA sequence of the gene fragment according to Bowler et al (1989) using site directed mutagenesis as described in Example 3: i. the AA nucleotides located upstream at position -2 and -1 of the ATG initiation codon are changed to CC nucleotides creating a Nco I site at the initiation codon and yielding the following nucleotide sequences:

- CCATGGCACTAC

NcoI

ii. the T,TCG,CTC nucleotides located immediately downstream of the processing site of the transit peptide are changed to C,TGC,AGC, creating a Pst I site behind the processing site and yielding the following nucleotide s quences:

```
             |  L   Q   T   F   S   L
CTC,CGC,GGC, |  TTG,CAG,ACC,TTT,TCG,CTC
CTC,CGC,GGC, |  TTG,CAG,ACC,TTC,TGC,AGC
             ↓                  PstI
```

in which the arrow indicates the processing site of the transit peptide sequence and the upper line the aminoacid sequence corresponding with the Mn-SOD coding sequence; the Nco I-Pst I fragment is also fused in frame with a gene encoding the Eco RI restriction endonuclease from E. coli (Greene et al (1981) J. Biol. Chem. 256 , 2143-2153; Botterman and Zabeau (1985) Gene 37 , 229-239) and capable of recognition and cleavage of the target sequence GAATTC on a double stranded DNA, as found in pMG104; and

b) the 3′ end of the NOS gene of Example 4; and

5. a gene encoding the Eco RI methylase under the control of its natural promoter (Botterman and Zabeau, 1985), which is capable of inhibiting the activity of Eco RI in E. coli or Agrobacterium , in order to overcome potential leaky expression of the Eco RI gene in microorganisms, this gene being inserted into the vector fragment outside the border sequences.

Each pMG105 is a binary type T-DNA vector containing, within the border sequences, three chimeric sequences: PSSU-sfr and PNOS-NPTII which are marker DNAs under the control of respectively PSSU and PNOS as second promoters; and PSTMG-transit peptide-EcoRI endonuclease gene which is a female-sterility DNA having the PSTMG as a first promoter and a transit peptide-encoding sequence between them. Expression of the female-sterility DNA under the control of the PSTMG promoter selectively in style and/or stigma cells will produce a restriction endonuclease which will be targeted into the mitochondria of the style and/or stigma cells and cleave the double stranded DNA at the GAATTC sites in such cells. This will lead to the death of these cells.

Example 11 - Introduction of each chimaeric DNA sequence of Example 10 into tobacco and alfalfa

As described in Example 5, each pMG104 and pMG105 (from Example 10) is mobilized from E. coli into separate Agrobacteria C58C1 Rif^R carrying pMP90. The resulting strains, harboring pMG104 with pMP90 and pMG105 with pMP90, are used to transform tobacco and alfalfa following the procedures described in Examples 5. That the Eco RI endonuclease genes are not expressed in transformed herbicide- and kanamycin-resistant calli, shoots and roots is shown by their growth.

The transformed plants are transferred into the greenhouse and grown in soil until they flower. The flowers of both the tobacco and alfalfa are examined, and essentially the same phenotypes are observed for the transformed plants as are observed in the transformed plants described in Example 5 (i.e., no normal style-stigma formation). The transformed plants are female-sterile.

Needless to say, this invention is not limited to the transformation of any specific plant(s). The invention relates to any plant, the nuclear genome of which can be transformed with a female-sterility DNA under the control of a first promoter that can direct expression of the female-sterility DNA selectively in cells of the flowers, particularly a female organ thereof, or the seeds or the embryos of the plant, whereby the plant can be both self-pollinated and cross-pollinated.

Also, this invention is not limited to the specific plasmids and vectors described in the foregoing Examples, but rather encompasses any plasmids and vectors containing the female-sterility DNA under the control of the first promoter.

Furthermore, this invention is not limited to the specific PSTMG promoters, described in the foregoing Examples, but rather encompasses any DNA sequence encoding a promoter capable of directing expression of the female-sterility DNA selectively in cells of flowers, particularly one or more female organs thereof, and/or seeds and/or embryos of the plant.

In addition, this invention is not limited to the specific female-sterility DNAs described in the foregoing

Examples but rather encompasses any DNA sequence encoding a first RNA, protein or polypeptide which significantly disturbs adversely the metabolism, functioning and/or development of a cell of a flower, seed or embryo of a plant in which it is produced, under the control of the first promoter.

Also, this invention is not limited to the specific marker DNAs described in the foregoing Examples but rather encompasses any DNA sequence encoding a second RNA, protein or polypeptide which confers on at least a specific plant tissue or specific plant cells, in which such DNA sequence is expressed, a distinctive trait compared to such a specific plant tissue or specific plant cells in which such DNA sequence is not expressed.

Claims

1. A cell of a plant, the nuclear genome of which contains, stably integrated therein, a foreign DNA sequence, preferably a foreign chimaeric DNA sequence, characterized by:

(a) a female-sterility DNA encoding a first RNA, protein or polypeptide which, when produced or overproduced in a cell of a flower, particularly a female organ thereof, or a seed or an embryo of said plant, disturbs significantly the metabolism, functioning and/or development of said cell; and

(b) a first promoter capable of directing expression of said female-sterility DNA selectively in cells of the flowers, particularly a female organ thereof, or the seeds or the embryos of said plant, preferably in style, stigma, ovule, septum, seed coat, endosperm, embryo axis or embryo cotyledon cells; said female-sterility DNA being in the same transcriptional unit as, and under the control of, said first promoter; provided that said foreign DNA sequence is preferably present in homozygous form in the nuclear genome of said transformed plant cell if said first promoter is capable of directing expression of said female-sterility DNA selectively: i) in cells of female gametes, such as ovules, especially those female gamete cells which have undergone meiosis, or ii) in cells derived from said female gamete cells, such as cells of seeds or embryos.

2. The cell of claim wherein said foreign DNA sequence is present in a homozygous form in the nuclear genome of said cell.

3. The cell of claim 1 wherein said first promoter is capable of directing expression of said female- sterility DNA selectively in cells of the flowers, particularly a female organ thereof, or the seeds of said plant, preferably in ovary, ovule, style, septum and/or seed coat cells.

4. The cell of anyone of claims 1-3 wherein said foreign DNA sequence also comprises, preferably in the same genetic locus as said female-sterility DNA:

(c) a marker DNA encoding a second RNA, protein or polypeptide which, when present at least in a specific tissue or in at least specific cells of said plant, renders said plant easily separable from other plants which do not contain said second RNA, protein or polypeptide at least in said specific tissue or specific cells; and

(d) a second promoter capable of directing expression of said marker DNA at least in said specific tissue or specific cells; said marker DNA being in the same transcriptional unit as, and under the control of, said second promoter.

5. The cell of anyone of claims 1-4 wherein said foreign DNA sequence further comprises:

(e) a first DNA encoding a transit peptide capable of transporting said first protein or polypeptide into a chloroplast or mitochondria of said cell of a flower, seed or embryo of said plant; said first DNA being in the same transcriptional unit as said female-sterility DNA and said first promoter and between said female-sterility DNA and said first promoter; and/or

f) a second DNA encoding a transit peptide capable of transporting said second protein or polypeptide into a chloroplast or mitochondria of at least said specific tissue or specific cells; said second DNA being in the same transcriptional unit as said marker DNA and said second promoter and between said marker DNA and said second promoter.

6. The cell of anyone of claims 1-5 wherein said foreign DNA sequence further comprises:

g) a third DNA encoding a secretory signal peptide capable of secreting said first protein or polypeptide outside said cell of a flower, seed or embryo of said plant; said third DNA being in the same transcriptional unit as said female-sterility DNA and said first promoter and located between said female-sterility DNA and said first promoter; and/or

h) a fourth DNA encoding a secretory signal peptide capable of secreting said second protein or polypeptide outside at least said specific tissue or specific cells; said fourth DNA being in the same transcriptional unit as said marker DNA and said second promoter and located between said marker DNA and said second promoter.

7. The cell of anyone of claims 1-6 wherein said female-sterility DNA encodes: an RNase, particularly RNase T1 or Barnase; a DNase, especially an endonuclease, particularly Eco RI; a protease, especially a papain, particularly papain Zymogen or papain active protein: a glucanase; a lipase, particularly phospholipase A_2; a lipid peroxidase; a cell wall inhibitor; a bacterial toxin; or a ribozyme, particularly a ribozyme against mRNA encoded by any of the STMG-type genes, by the KTI3 gene, by a gene encoding a seed-specific storage protein, such as a 2S albumin, or by a gene corresponding to cDNA clone pMON9608; or alternatively, wherein said female sterility DNA: encodes a ribonuclease, particularly T_2 or Rh; encodes a glycoprotein encoded by the S1, S2, S3, S6 or S7 alleles, particularly of Nicotiana alata ; or is an anti-sense DNA, particularly an antisense DNA of an STMG-type gene, the KTI3 gene, a gene encoding a seed-specific storage protein, such as a 2S albumin, or a gene corresponding to cDNA clone pMON9608.

8. The cell of anyone of claims 1-6 wherein said female-sterility DNA encodes an enzyme which catalyzes the synthesis of a phytohormone, particularly an enzyme encoded by gene 1, gene 2 or gene 4 of Agrobacterium T-DNA or alternatively the enzymes encoded by gene 1 and gene 2 of Agrobacterium T-DNA.

9. The cell of anyone of claims 1-6 wherein said female-sterility DNA encodes a viral dependent RNA polymerase, particularly a TNV replicase, under the control of said first promoter; and a negative strand of a gene encoding said first protein or polypeptide; said gene being in the same transcriptional unit as, and under the control of, a promoter, such as another first promoter, capable of directing expression of said negative strand in the cell; said gene also being fused at its 3′ end to a viral subgenomic promoter, such as the TNV subgenomic promoter, which is specifically recognized by said viral dependent RNA polymerase.

10. The cell of anyone of claims 4-9, wherein said marker DNA is: an herbicide resistance gene, particularly an sfr or sfr v gene; a gene encoding a modified target enzyme for an herbicide having lower affinity for the herbicide, particularly a modified 5-enolpyruvylshikimate-3 phosphate synthase as a target for glyphosate or a modified glutamine synthetase as a target for a glutamine synthetase inhibitor such as phosphinotricine; a gene encoding a protein or a polypeptide conferring a color to at least said specific tissue or specific cells, particularly the gene A1 or the GUS gene; a gene encoding a protein or a polypeptide conferring a stress tolerance to said plant, particularly the gene encoding Mn-superoxide dismutase; or a gene encoding a protein or a polypeptide conferring a disease or pest resistance, particularly a gene encoding a Bacillus thuringiensis endotoxin that confers insect resistance or a gene encoding a bactericidal peptide that confers a bacterial resistance.

11. The cell of anyone of claims 1-10, wherein said first promoter is: PSTMG07, PSTMG08, PSTMG4B12 or PSTMG3C9; a promoter derived from a self-incompatibility gene, such as an S-gene; the promoter of the KTI3 gene; a promoter of a gene encoding a seed-specific storage protein, such as a PAT2S promoter; a promoter of a gene corresponding to cDNA clone pMON9608; or a promoter of DNA coding for i) a style-stigma specific, ii) embryo axis-specific, iii) seed-specific or iv) ovule-specific mRNA which is hybridizable respectively to i) an STMG-type gene or S-gene, ii) the KTI3 gene, iii) an AT2S gene or iv) cDNA clone pMON9608.

12. The cell of anyone of claims 4-11, wherein said second promoter is: a constitutive promoter, particularly a 35S promoter, a 35S′3 promoter, a PNOS promoter or a POCS promoter; a wound-inducible promoter, particularly a TR1′ or TR2′ promoter; a promoter which directs gene expression selectively in plant tissue having photosynthetic activity, particularly an SSU promoter; or a promoter which directs gene expression selectively in leaf cells, petal cells or seed cells, particularly seed coat cells.

13. The cell of anyone of claims 1 and 3-12 that comes from a culture of diploid or haploid cells of said plant, which cells have been transformed with said foreign DNA sequence; and wherein the cell can be used to regenerate said plant as a homozygous plant; and particularly wherein said first promoter is capable of directing expression of said female-sterility DNA selectively: i) in cells of female gametes, such as ovules, particularly after meiosis of said female gamete cells, or ii) in cells derived from said female gamete cells, such as cells of seeds or embryos.

14. A vector suitable for transforming a cell of a plant, particularly a plant capable of being infected with Agrobacterium , comprising said foreign DNA sequence of anyone of claims 1-13, particularly a pMG100, a pMG101, a pMG102, a pMG103, a pMG104 or a pMG105.

15. A process for producing a female-sterile plant and reproductive material of said plant, having said foreign DNA sequence of anyone of claims 1-13 stably integrated into the nuclear genome of their cells, whereby said female-sterility DNA is capable of being expressed selectively in cells of a flower, particularly a female organ, or a seed or an embryo of said plant to produce said first RNA, protein or polypeptide in said cells and whereby said marker DNA can be expressed in at least said specific tissue or specific cells of said plant to render said plant separable from non-transformed plants, characterized by the non-biological

steps of: a) transforming a cell of said plant by introducing said foreign DNA sequence into the nuclear genome of said cell; and then b) regenerating said plant and reproductive materials from said cell.

16. A plant cell culture containing the plant cell of anyone of claims 1-13.

17. A plant containing the plant cell of anyone of claims 1-13.

18. A fruit of the plant of claim 17, particularly a seedless fruit.

19. A process for producing a hybrid seed or a seedless fruit, characterized by the steps of cross-pollinating:

a) a female-sterile male-fertile plant that contains the foreign DNA sequence of anyone of claims 1-13, preferably including both said second promoter and said marker DNA, especially said marker DNA conferring a resistance to an herbicide, particularly an sfr or sfr v gene, stably integrated in the nuclear genome of the cells of said female-sterile plant; with b) a male-sterile female-fertile plant, the nuclear genome of which contains, stably integrated therein: i) a male-sterility DNA encoding a third RNA, protein or polypeptide which, when produced or overproduced in stamen cells of said plant, disturbs significantly the metabolism, functioning and/or development of said stamen cells and which is in the same transcriptional unit as, and under the control of, a promoter capable of directing expression of said male-sterility DNA selectively in said stamen cells; and ii) optionally, in the same genetic locus, another marker DNA and a promoter thereof, preferably said marker DNA and said second promoter.

20. Hybrid seed obtained by the process of claim 19, wherein said first promoter directs expression of said female-sterility DNA selectively in cells of flowers or embryos, particularly flowers.

21. A hybrid plant obtained by growing the hybrid seed of claim 20.

22. Seedless fruit obtained by the process of claim 19, wherein said first promoter directs expression of said female-sterility DNA selectively in cells of seeds.

23. The first promoter of claim 11, particularly PSTMG07, PSTMG08, PSTMG4B12 or PSTMG3C9.

24. A foreign chimaeric DNA sequence of anyone of claims 1-13, wherein said female-sterility DNA is not naturally found under the control of said first promoter and/or is not naturally found in the same genetic locus as said marker DNA, particularly wherein said first DNA or said third DNA is not naturally found between said first promoter and said female-fertility DNA.

25. In a process for producing a plant and its reproductive material, such as seeds, or for producing fruit of said plant, including a foreign genetic material stably integrated in the nuclear genome thereof and capable of expressing therein an RNA, protein or polypeptide, comprising the non-biological steps of: a) producing transformed cells or tissue of said plant including said foreign genetic material from starting plant cells or plant tissue not expressing said RNA, protein or polypeptide, b) producing regenerated plants or reproductive material or both of said plant from said transformed plant cells or plant tissue including said foreign genetic material, and c) optionally, biologically replicating said regenerated plants or reproductive material or both; wherein said step of producing said transformed plant cells or plant tissue including said foreign genetic material is characterized by: transforming the nuclear genome of said starting plant cells or plant tissue with a foreign DNA sequence of anyone of claims 1-13, as well as regulatory elements which are capable of enabling the expression of said foreign DNA sequence in said plant cells or plant tissue, to cause the stable integration of said foreign DNA sequence in transformed plant cells or plant tissue, as well as in plants and reproductive material produced therefrom throughout subsequent generations.

Figure 1 A

```
                9                18                27                36
GAA TTC CCA TTG CCT TTC GAA TTG CCA CCA GCG GAG ATC
Glu Phe Pro Leu Pro Phe Glu Leu Pro Pro Ala Glu Ile

         45                54                63                72
CCA TTG CCG GAG ATC CCA TTG CCT TTC GAT GGG CCT ACA
Pro Leu Pro Glu Ile Pro Leu Pro Phe Asp Gly Pro Thr

 81                90                99               108               117
TTC GTG CTA CCG CCA CCA TCA CCA CCA CCA CCT CCA TCG
Phe Val Leu Pro Pro Pro Ser Pro Pro Pro Pro Pro Ser

              126               135               144               153
TCA CCA TCT CCA TCT CCA GCA AAG CAA TCA CCA CCA CCT
Ser Pro Ser Pro Ser Pro Ala Lys Gln Ser Pro Pro Pro

        162               171               180               189
CCT CGG GCA CCA TCA CCA TCA CCA GCT ACT CAG CCA CCT
Pro Arg Ala Pro Ser Pro Ser Pro Ala Thr Gln Pro Pro

198               207               216               225               234
ATA AAG CAA CCG CCA CCA CCA AGT GCT AAG AAA TCT CCT
Ile Lys Gln Pro Pro Pro Pro Ser Ala Lys Lys Ser Pro

              243               252               261               270
CCG CCA CCA GTT GCT TAT CCA CCA GTT ATG GCA CCA TCT
Pro Pro Pro Val Ala Tyr Pro Pro Val MET Ala Pro Ser

        279               288               297               306
CCA TCA CCG GCT GCT GAG CCA CCT ATT ATA GCA CCA TTT
Pro Ser Pro Ala Ala Glu Pro Pro Ile Ile Ala Pro Phe
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Figure 1 A (cont. 1)

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315         324         333         342         351
CCA TCA CCA ACA GCG AAT CTA CCC CTT ATT CCC CGT CGA
Pro Ser Pro Thr Ala Asn Leu Pro Leu Ile Pro Arg Arg

        360         369         378         387
CCA GCA CCA CCA GTA GTT AAG CCG CTT CCA CCT TTG GGG
Pro Ala Pro Pro Val Val Lys Pro Leu Pro Pro Leu Gly

    396         405         414         423
AAG CCC CCT ATC GTC AAT GGC CTT GTT TAT TGT AAA TCC
Lys Pro Pro Ile Val Asn Gly Leu Val Tyr Cys Lys Ser

432         441         450         459         468
TGC AAC AGC TAT GGG TTC CCC ACT CTG CTC AAC ACC TCC
Cys Asn Ser Tyr Gly Phe Pro Thr Leu Leu Asn Thr Ser

        477         486         495         504
CTA CTC CCA GGA GCT GTT GTG AAA CTA GTT TGC TAC AAC
Leu Leu Pro Gly Ala Val Val Lys Leu Val Cys Tyr Asn

    513         522         531         540
GGA AAG AAA ACA ATG GTT CAA TCG GCG ACG ACA GAC AAC
Gly Lys Lys Thr MET Val Gln Ser Ala Thr Thr Asp Asn

549         558         567         576         585
AAA GGT GAG TTT CGG ATC ATT CCC AAA TCA TTA ACC AGA
Lys Gly Glu Phe Arg Ile Ile Pro Lys Ser Leu Thr Arg

        594         603         612         621
GCA GAT GTT GGC AAG TGC AAG TTA TAT TTA GTG AAA TCA
Ala Asp Val Gly Lys Cys Lys Leu Tyr Leu Val Lys Ser
```

Figure 1 A (cont. 2)

```
     630         639         648         657
CCA AAT CCA AAT TGC AAT GTC CCA ACA AAT TTC AAT GGT
Pro Asn Pro Asn Cys Asn Val Pro Thr Asn Phe Asn Gly

666         675         684         693         702
GGA AAA TCT GGT GGT TTA TTG AAG CCT CTC CTA CCA CCT
Gly Lys Ser Gly Gly Leu Leu Lys Pro Leu Leu Pro Pro

        711         720         729         738
AAA CAA CCG ATT ACC CCT GCC GCT GTC CCT CTA TCT GAT
Lys Gln Pro Ile Thr Pro Ala Ala Val Pro Leu Ser Asp

    747         756         765         774
TTA TAT GGT GTT GGA CCT TTT ATA TTT GAA GCC TCC AGC
Leu Tyr Gly Val Gly Pro Phe Ile Phe Glu Ala Ser Ser

783         792         801         810         819
AAA ATG CCA TGC GAT AAG AAT TGA GCT CCT CAT TAC TAG
Lys MET Pro Cys Asp Lys Asn  .  Ala Pro His Tyr  .

        828         837         846         855
AGC GAT AAT GTA TAA GAG CAT GAG TTT GTG ACG GAA ATT
Ser Asp Asn Val  .  Glu His Glu Phe Val Thr Glu Ile

    864         873         882         891
ATT TTT TTC TTT TTT GTT CTA TAG TTT ATA CAA GGA GAC
Ile Phe Phe Phe Phe Val Leu  .  Phe Ile Gln Gly Asp

900         909         918         927         936
AGA AAA CTT TGT ATC ACT ATA CAG AAA TCA AAT GAG TCG
Arg Lys Leu Cys Ile Thr Ile Gln Lys Ser Asn Glu Ser
```

Figure 1 A (cont. 3)

```
        945         954         963
CAA AAG TCA AAA TCG AAT TTA TGA AAA
Gln Lys Ser Lys Ser Asn Leu  .  Lys
```

Figure 1 B

```
             11          20          29          38
GA ATT CCG GCT TTT ACA TCA GTA AAG ATC CTA GTG CTC
   Ile Pro Ala Phe Thr Ser Val Lys Ile Leu Val Leu

         47          56          65          74
ATA CAA GTT TCA GTT TTA GCA CTC AGC TCA TTC TCA GAG
Ile Gln Val Ser Val Leu Ala Leu Ser Ser Phe Ser Glu

     83          92         101         110
CTT AGC TTT GGT AAA GGA ATT GAA AGC TCG TCA TTA GAC
Leu Ser Phe Gly Lys Gly Ile Glu Ser Ser Ser Leu Asp

119         128         137         146         155
AAA GGA CAA CAC CAT CCA ATC TTC TCA ACA GTT CAC TTA
Lys Gly Gln His His Pro Ile Phe Ser Thr Val His Leu

        164         173         182         191
TTC TTT GGA AAG TCT CCC AAG AAA AGC CCC TCT AGC CCT
Phe Phe Gly Lys Ser Pro Lys Lys Ser Pro Ser Ser Pro

    200         209         218         227
ACA CCG GTA AAC AAG CCA TCA CCA TCA CCA CCA CCA CAG
Thr Pro Val Asn Lys Pro Ser Pro Ser Pro Pro Pro Gln

236         245         254         263         272
GTT AAG TCA TCC CTT CCG CCG CCT GCT AAG TCA CCA CCG
Val Lys Ser Ser Leu Pro Pro Pro Ala Lys Ser Pro Pro

        281         290         299         308
CCG CCA CCA GCT AAG TCA CCA CCT CCT CTG CTG CCT CCA
Pro Pro Pro Ala Lys Ser Pro Pro Pro Leu Leu Pro Pro
```

Figure 1 B (cont. 1)

```
    317         326         335         344
CCA CCA TCT CAA CCA CCA AAA CAA CCA CCT CCA CCT CCG
Pro Pro Ser Gln Pro Pro Lys Gln Pro Pro Pro Pro Pro

353         362         371         380         389
CCG CCA CCA GCA AAG CAA CCA CCA TCT GCT AAG CCA CCT
Pro Pro Pro Ala Lys Gln Pro Pro Ser Ala Lys Pro Pro

        398         407         416         425
ATT AAA CCT CCA TCT CCG TCA CCG GCT GCT CAG CCA CCA
Ile Lys Pro Pro Ser Pro Ser Pro Ala Ala Gln Pro Pro

    434         443         452         461
GCA ACG CAA CGA GCA ACA CCA CCA CCG GCA ATG CAA CGG
Ala Thr Gln Arg Ala Thr Pro Pro Pro Ala MET Gln Arg

470
GCA CC
Ala
```

Figure 2 A

```
          9           18          27          36
GCC CTG TAG CGG CAT TAA GCG CGG CGG GTG TGG TGG TTA
Ala Leu  .  Arg His  .  Ala Arg Arg Val Trp Trp Leu

     45          54          63          72
CGC GCA GTG ACC GCT ACA CTT GCC AGC GCC CTA GCG CCC
Arg Ala Val Thr Ala Thr Leu Ala Ser Ala Leu Ala Pro

 81          90          99         108         117
GCT CCT TTC GCT CTT CTC ATT CTC ATC ATC CTC ACT CTT
Ala Pro Phe Ala Leu Leu Ile Leu Ile Ile Leu Thr Leu

        126         135         144         153
TCT AGC ACA CCA ATT ACC ACA ATG TCT ATA CCC GAG ACA
Ser Ser Thr Pro Ile Thr Thr MET Ser Ile Pro Glu Thr

    162         171         180         189
AAC CGT AGA AAT GCA ACT ACA AAC TCT TAC ACC GAT GTT
Asn Arg Arg Asn Ala Thr Thr Asn Ser Tyr Thr Asp Val

198         207         216         225         234
GCT CTT TCT GCG CGA AAA GGT GCA TTT CCT CCT CCC AGA
Ala Leu Ser Ala Arg Lys Gly Ala Phe Pro Pro Pro Arg

        243         252         261         270
AAG CTA GGA GAA TAC TCG ACA AAT TCT ACC GAC TAC AAC
Lys Leu Gly Glu Tyr Ser Thr Asn Ser Thr Asp Tyr Asn

    279         288         297         306
TTG ATC TGC AAA ACT TGC AAG AGA TTA TCG GAA CGC AAT
Leu Ile Cys Lys Thr Cys Lys Arg Leu Ser Glu Arg Asn
```

Figure 2 A (cont. 1)

```
315         324         333         342         351
ACA TGT TGT TTC AAC TAC AGT TGT GTT GAT GTG TCC ACC
Thr Cys Cys Phe Asn Tyr Ser Cys Val Asp Val Ser Thr

        360         369         378         387
AAC AGG TTC AAC TGT GGC TCC TGT GGC CTT GTC TGT AAC
Asn Arg Phe Asn Cys Gly Ser Cys Gly Leu Val Cys Asn

    396         405         414         423
CTT GGA ACG AGA TGC TGT GGT GGG ATC TGT GTG GAC ATC
Leu Gly Thr Arg Cys Cys Gly Gly Ile Cys Val Asp Ile

432         441         450         459         468
CAG AAA GAC AAT GGC AAT TGT GGC AAG TGT TCT AAT GTT
Gln Lys Asp Asn Gly Asn Cys Gly Lys Cys Ser Asn Val

        477         486         495         504
TGC TCT CCT GGT CAG AAG TGT TCA TTT GGG TTT TGT GAC
Cys Ser Pro Gly Gln Lys Cys Ser Phe Gly Phe Cys Asp

    513         522         531         540
TAT GCC TAA GTA TAT TTT CCC TAT GTC TAG TAA TAA CCA
Tyr Ala  .  Val Tyr Phe Pro Tyr Val  .   .   .  Pro

549         558         567         576         585
GAG TCT GTG TAA GCC TGT CAA ATA ACT AAC TCC CCT GTC
Glu Ser Val  .  Ala Cys Gln Ile Thr Asn Ser Pro Val

        594         603         612         621
CCT AGG GTG AAA TGT TAC TCT AAT AAC GTT GGA GAT TTG
Pro Arg Val Lys Cys Tyr Ser Asn Asn Val Gly Asp Leu
```

Figure 2 A (cont. 2)

```
    630         639         648         657
CAT TCT GTG TTG TTT GTA GTA AGT TAT GGC TAG TAA TCT
His Ser Val Leu Phe Val Val Ser Tyr Gly  .   .  Ser

666         675         684         693         702
ATT TAA GGT GAC TTG GAA TAC ATA AAA AAA AAA AAA AAA
Ile  .  Gly Asp Leu Glu Tyr Ile Lys Lys Lys Lys Lys

        711         720         729         738
AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA
Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys

    747
AAA TGC A
Lys Cys
```

Figure 2 B

```
             11       20       29       38
CC CTT GTT CTT TTT CAG CTT TCA GTT TTA TTA CTT AGC
   Leu Val Leu Phe Gln Leu Ser Val Leu Leu Leu Ser

        47       56       65       74
TCA TTC ACA GTA GTT CTT AGC CAG GAG GAA GAC ATT GGG
Ser Phe Thr Val Val Leu Ser Gln Glu Glu Asp Ile Gly

    83       92      101      110
GGT TGG TTT ACC ACC AAA CAT CAT GAC CAC CTT TCA CCA
Gly Trp Phe Thr Thr Lys His His Asp His Leu Ser Pro

119      128      137      146      155
GCT CAA GCT CCT AAG CCT CAC AAA GGC CAC CAC CAC CCC
Ala Gln Ala Pro Lys Pro His Lys Gly His His His Pro

        164      173      182      191
AAA CAT TCC CCA GCC CCT TCA CCA ACT AAG CCT CCC ACT
Lys His Ser Pro Ala Pro Ser Pro Thr Lys Pro Pro Thr

    200      209      218      227
TAT AGC CCA TCG AAA CCA CCA GTT AAA CCA CCG GTT AAA
Tyr Ser Pro Ser Lys Pro Pro Val Lys Pro Pro Val Lys

236      245      254      263      272
CCA CCA ACT AAG GCT CCC ACT TAT AGC CCA TCA AAA CCA
Pro Pro Thr Lys Ala Pro Thr Tyr Ser Pro Ser Lys Pro

        281      290      299      308
CCA GCT AAG CCA CCA GTT AAA CCA CCA ACA CCA ACA CCA
Pro Ala Lys Pro Pro Val Lys Pro Pro Thr Pro Thr Pro
```

Figure 2 B (cont. 1)

```
    317         326         335         344
TCA CCT TAT CCT GCT CCT GCT CCT ATT ACT AGG AAA CCT
Ser Pro Tyr Pro Ala Pro Ala Pro Ile Thr Arg Lys Pro

353         362         371         380         389
GTA GCA GTC CGT GGC CTT GTT TAC TGC AAG CCG TGC AAG
Val Ala Val Arg Gly Leu Val Tyr Cys Lys Pro Cys Lys

        398         407         416         425
TTT AGA GGG GTT AAA ACT CTA AAC CAA GCT TCC CCA CTC
Phe Arg Gly Val Lys Thr Leu Asn Gln Ala Ser Pro Leu

    434         443         452         461
CTG GGT GCG GTA GTG AAG CTA GTA TGC AAC AAC ACA AAG
Leu Gly Ala Val Val Lys Leu Val Cys Asn Asn Thr Lys

470         479         488         497         506
AAG ACA TTA GTG GAA CAG GGC AAG ACA GAC AAG AAT GGC
Lys Thr Leu Val Glu Gln Gly Lys Thr Asp Lys Asn Gly

        515         524         533         542
TTC TTC TGG ATC ATG CCC AAA TTC TTG TCC TCA GCA GCT
Phe Phe Trp Ile MET Pro Lys Phe Leu Ser Ser Ala Ala

    551         560         569         578
TAC CAC AAA TGC AAG GTG TTC TTG GTC TCA TCA AAC AAT
Tyr His Lys Cys Lys Val Phe Leu Val Ser Ser Asn Asn

587         596         605         614         623
ACT TAC TGT GAT GTC CCA ACA GAT TAC AAT GGT GGA AAA
Thr Tyr Cys Asp Val Pro Thr Asp Tyr Asn Gly Gly Lys
```

Figure 2 B (cont. 2)

```
        632         641         650         659
TCT GGT GCT TTG TTG AAA TAC ACC CCA CTT CCT AAG CCA
Ser Gly Ala Leu Leu Lys Tyr Thr Pro Leu Pro Lys Pro

    668         677         686         695
CCA GCA GCT ACT TCT CTC CCT GTT AAA CTC CCC ACA TTT
Pro Ala Ala Thr Ser Leu Pro Val Lys Leu Pro Thr Phe

704         713         722         731         740
GAT GTC TTC ACT GTT GGA CCT TTT GGT TTC GAA CCC TCA
Asp Val Phe Thr Val Gly Pro Phe Gly Phe Glu Pro Ser

        749         758         767         776
AAG AAG GTG CCA TGC AAA AAG TAA CTT GCA TGG GAA ATT
Lys Lys Val Pro Cys Lys Lys  .  Leu Ala Trp Glu Ile

    785         794         803         812
AGA AAG ATA GGA AGG AAA AAT TAA TTA TGT GTT GAA GAA
Arg Lys Ile Gly Arg Lys Asn  .  Leu Cys Val Glu Glu

821         830         839         848         857
AGA CGA TTA TGT ACC TGT TTC CTG TGT TCT TGT TAT TAT
Arg Arg Leu Cys Thr Cys Phe Leu Cys Ser Cys Tyr Tyr

        866         875         884         893
TTT ATT AAT AAA TGA AGC AAA GAG GAA AGA ACG TAG TTT
Phe Ile Asn Lys  .  Ser Lys Glu Glu Arg Thr  .  Phe

    902         911         920         929
TCT TGT TTT CCT ATT TTG TTT CTC TCT ATC AAA ACC CAA
Ser Cys Phe Pro Ile Leu Phe Leu Ser Ile Lys Thr Gln
```

Figure 2 B (cont. 3)

```
938                     947                     956                     965                     974
CAA GTA AAA TGG ATT TAT AAG TTT TTC TTC AAA AAA AAA
Gln Val Lys Trp Ile Tyr Lys Phe Phe Phe Lys Lys Lys

            983             992            1001            1010
AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA
Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys

    1019            1028            1037            1046
AAA AAA AAA AAA AAA AAA AAA AAT GCA GGT CGA
Lys Lys Lys Lys Lys Lys Lys Asn Ala Gly Arg
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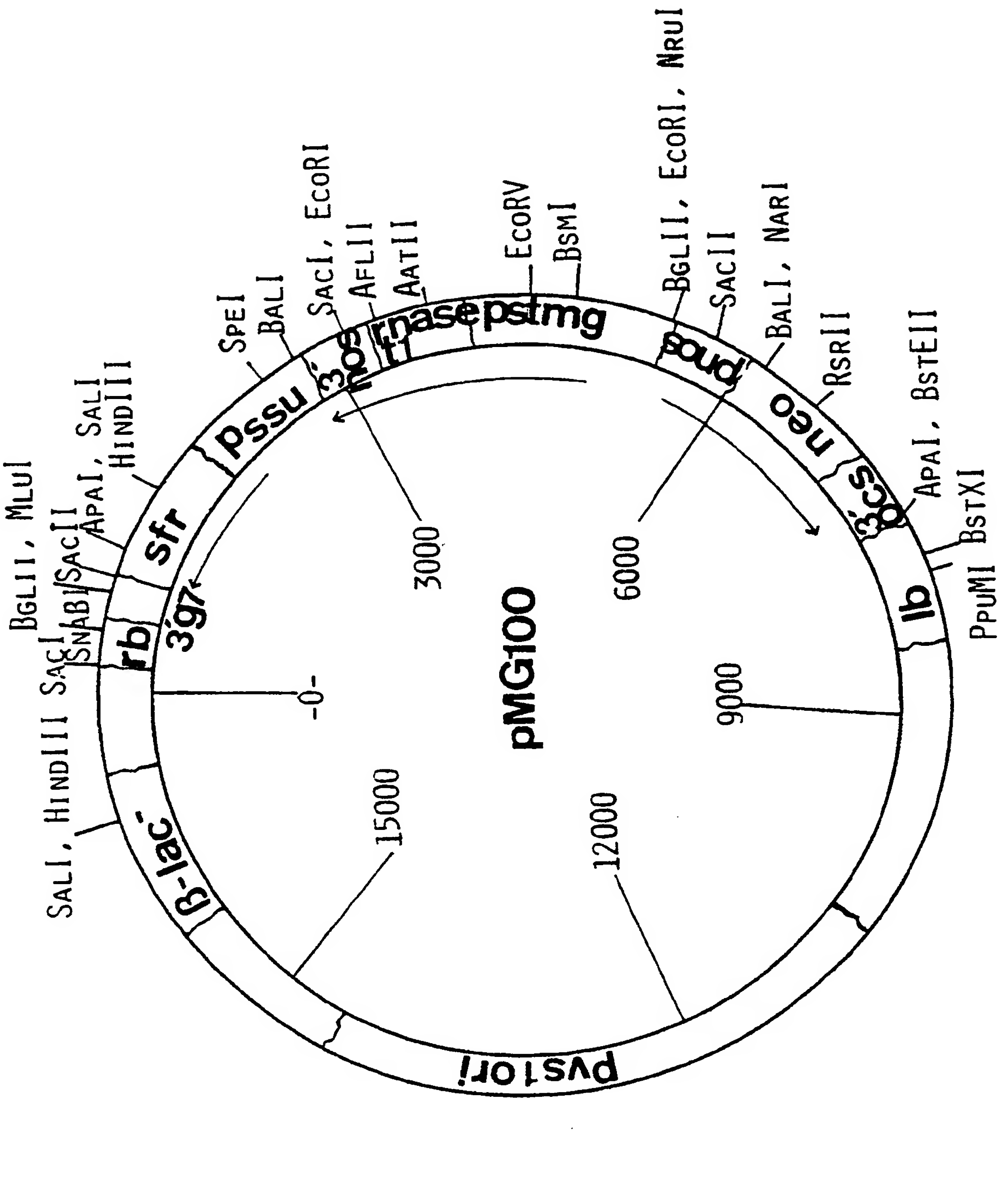
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β-lac⁻
rb
sfr
3'g7
SpeI
BalI
SacI, EcoRI
AflII
AatII
EcoRV
BsmI
BglII, EcoRI, NruI
SacII
BalI, NarI
RsrII
ApaI, BstEII
BstXI
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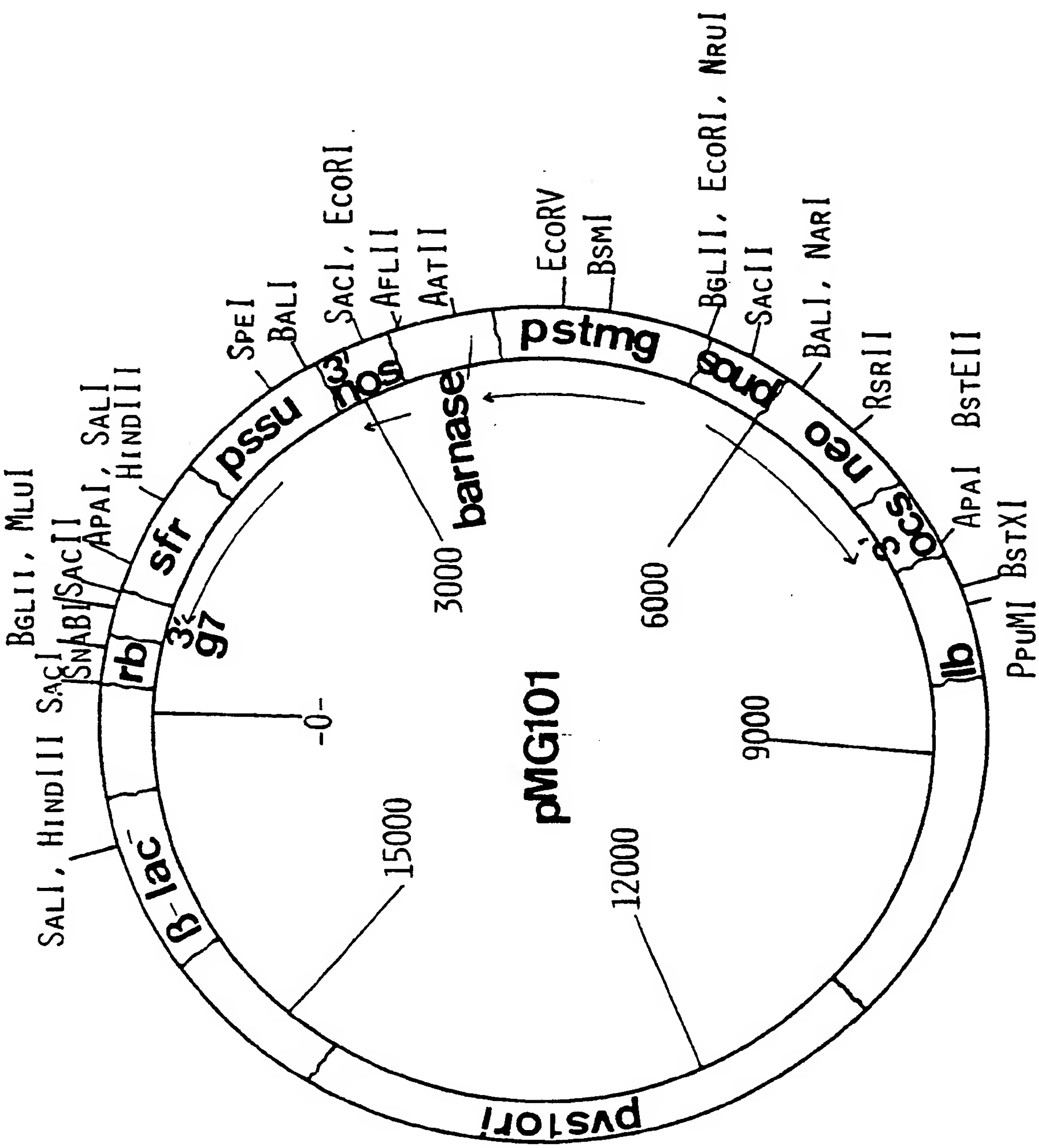
SnaBI
BglII, MluI
SacII
ApaI, SalI
HindIII

FIG.3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pvs1ori
pnos
neo
3' ocs
lb
SalI, HindIII
SacI
SnaBI
BglII, MluI
SacII
ApaI
ApaI, SalI
HindIII
SpeI
BalI
SacI, EcoRI
AflII
AatII
EcoRV
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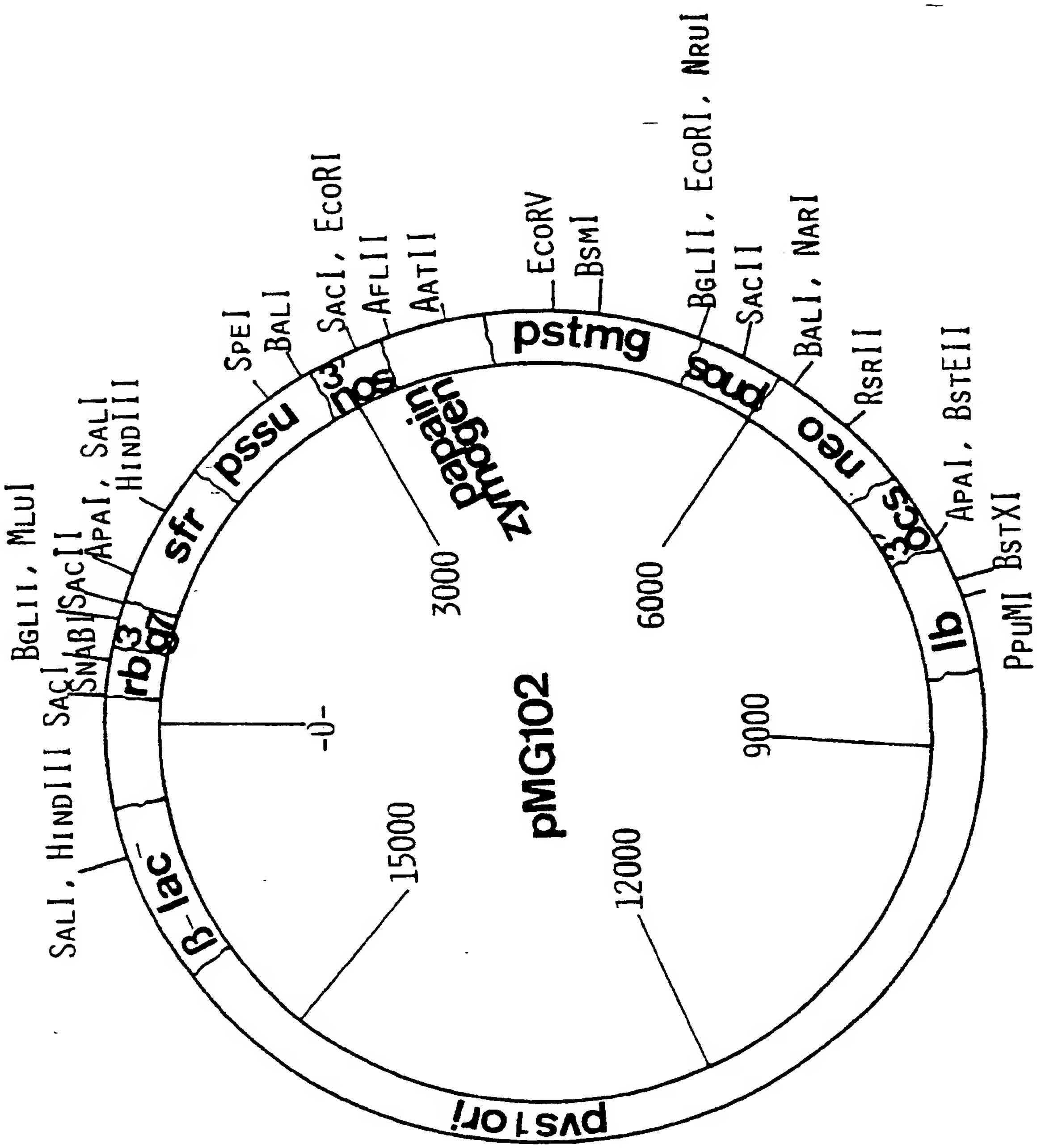
BstEII
ApaI
BstXI
PpuMI

FIG. 4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β-lac
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3'g7
sfr
pssu
3'nos
zymogen papain
SpeI
BalI
SacI, EcoRI
AflII
AatII
EcoRV
BsmI
BglII, EcoRI, NruI
SacII
BalI, NarI
RsrII
ApaI, BstEII
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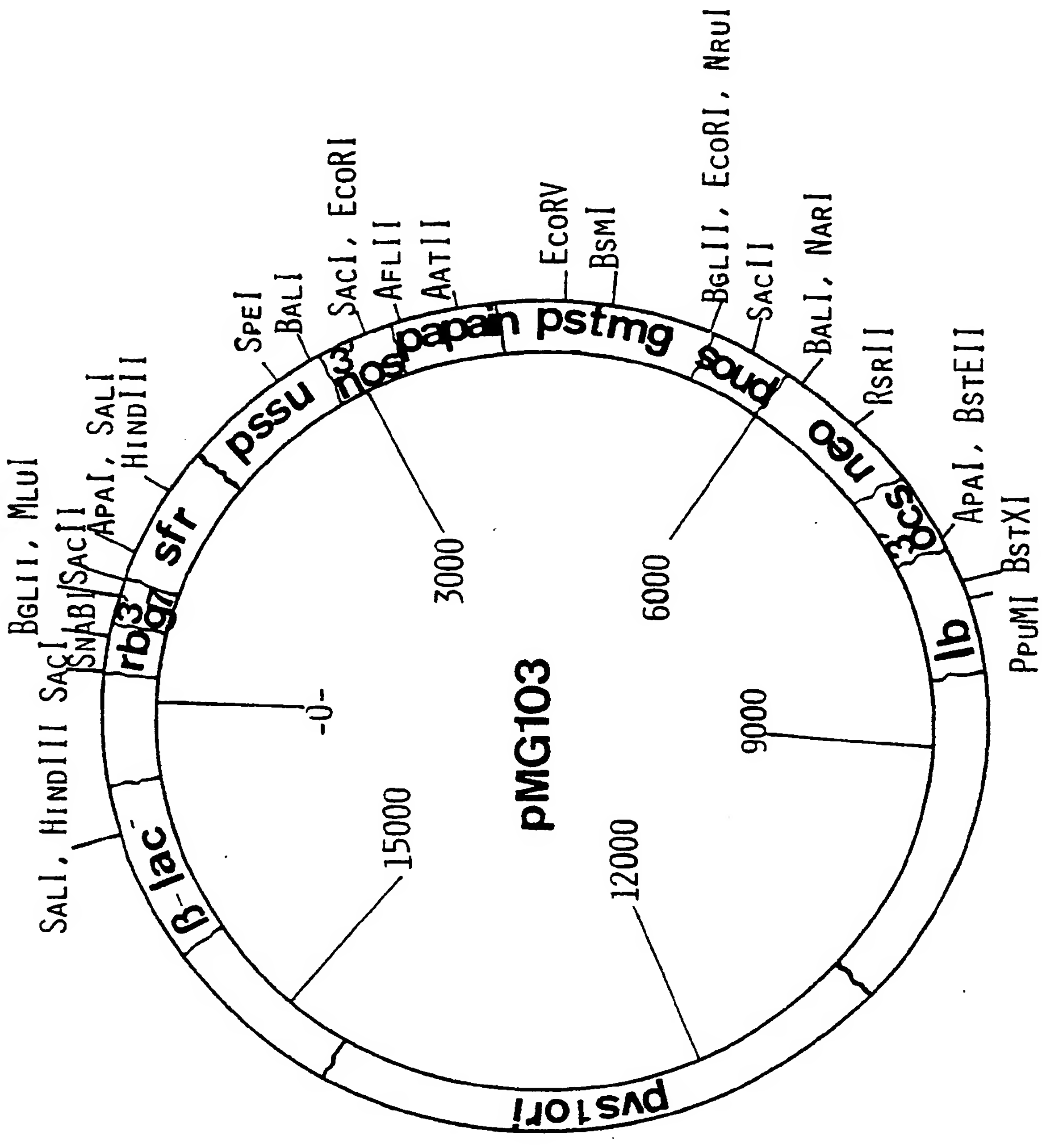
SnaBI
SacI
SalI, HindIII SacI

FIG.5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HindIII
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AflII
AatII
EcoRV
BsmI
BglII, EcoRI, NruI
SacII
BalI, NarI
RsrII
ApaI, BstEII
BstXI
PpuMI
β-lac
rb
3'
g7
sfr
pssu
3' nos
papain
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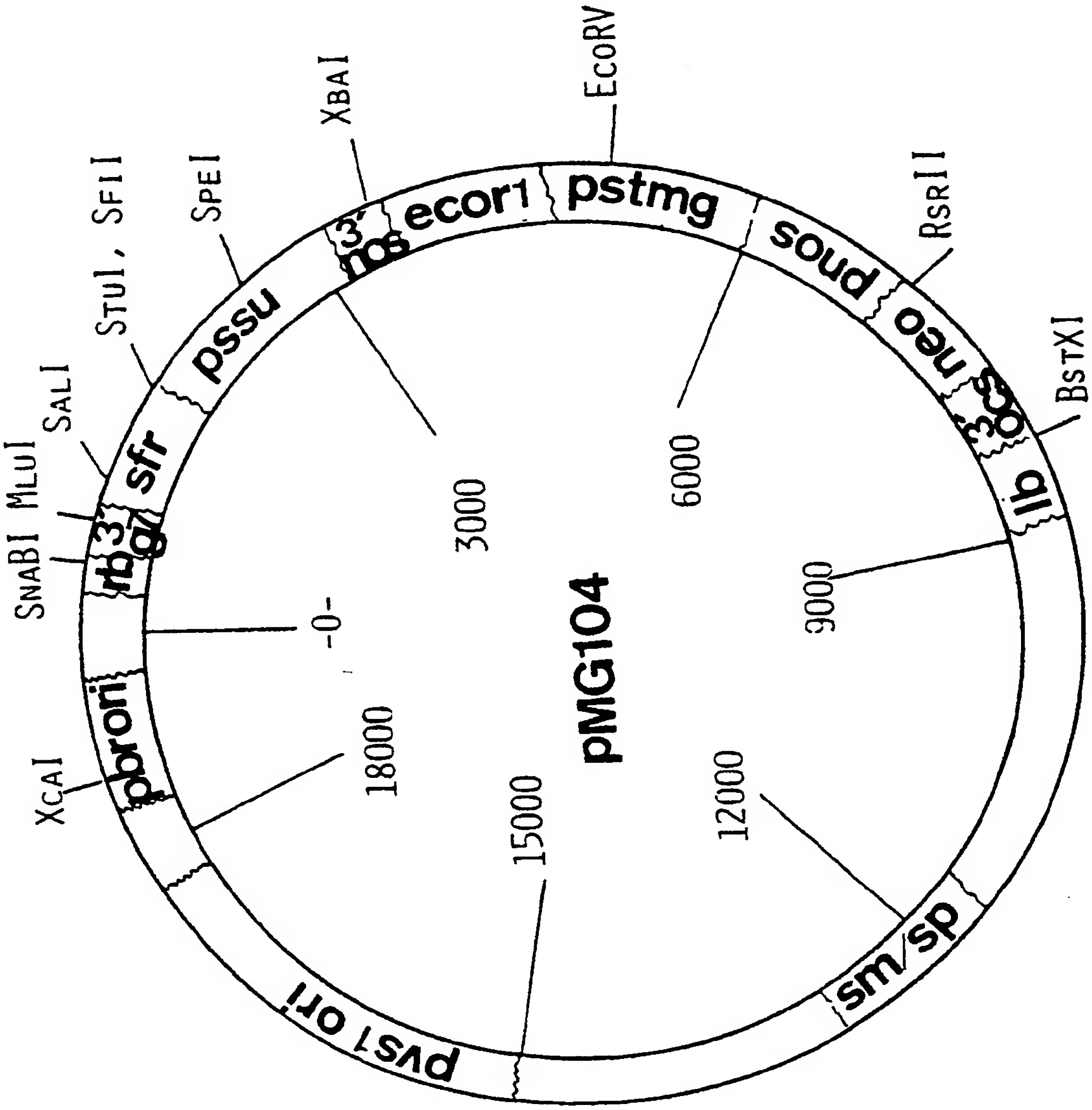
9000
12000
15000

FIG. 6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3' g7
rb
pbrori
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3' ocs
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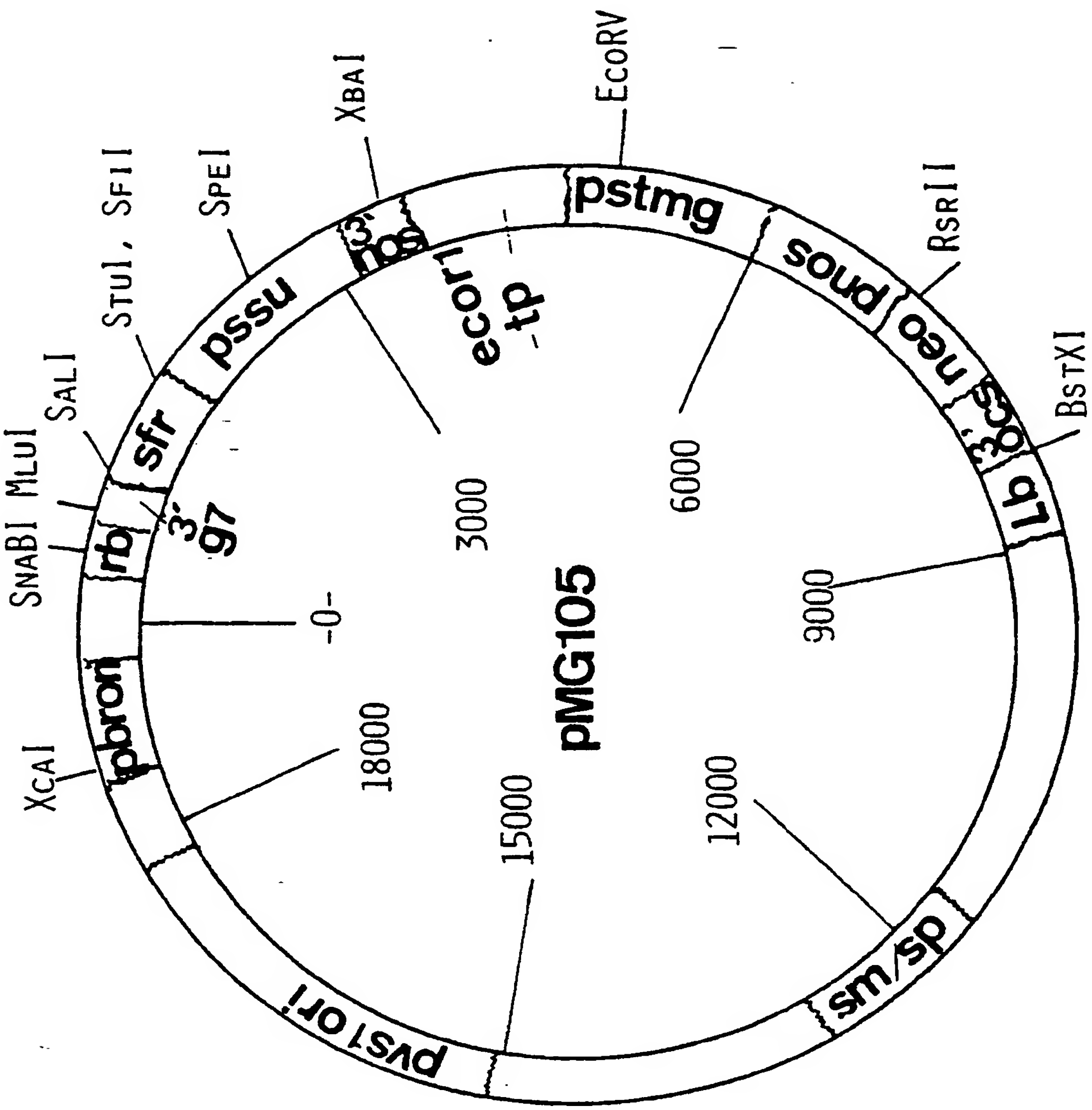
6000
9000
12000
15000
18000

FIG. 7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RsrII
BstXI
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neo
3' ocs
Lb
sm/sp
pvs1ori
pbron
rb
sfr
pssu
3' nos
ecor1
-tp
3' g7
-0-
3000
6000
9000
12000
15000
18000

FIG. 8

European Patent Office

EUROPEAN SEARCH REPORT

Application Number

EP 90 40 2196

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
O,X	J. CELL. BIOCHEM. SUPPL. 12C, 1988, UCLA SYMPOSIA ON MOLECULAR & CELLULAR BIOLOGY, ABSTRACTS 17TH ANNUAL MEETINGS, The Molecular Basis of Plant developement, 28th February - 10th April 1988, page 152, abstract no. L 051, Alan R. Liss, Inc., New York, US; H.J. KLEE et al.: "Manipulation of endogenous auxin and cytokinin levels in transgenic plants" * Abstract L 051 *	1-3,8,11, 13,14,23, 24	C 12 N 15/82 C 12 N 15/29 C 12 N 5/10 A 01 H 5/00
P,X	EP-A-0 343 947 (LUBRIZOL) * Page 4, lines 34-39 *	11,23	
X	THEOR. APPL. GENET., vol. 77, 1989, pages 320-324, Springer-Verlag; R. BERNATZKY et al.: "A nuclear sequence associated with self-incompatibility in Nicotiana alata has homology with mitochondrial DNA" * Page 321, left-hand column, paragraph 1: "Results" *	23	
Y	THE PLANT CELL, vol. 1, January 1989, pages 15-24, American Society of Plant Physiologists; C.S. GASSER et al.: "Isolation of tissue-specific cDNAs from tomato pistils" * Page 22, left-hand column, lines 1-17 *	1-4,7,8, 11,12,13, 15-17,23, 24,25	**TECHNICAL FIELDS SEARCHED (Int. Cl.5)**
Y	JOURNAL OF CELLULAR BIOCHEMISTRY, supplement 13D, 27th March - 7th April 1987, page 312, abstract no. M349, Alan R. Liss, Inc., New York, US; D. TWELL et al.: "Pollen-specific expression directed by chimaeric genes in transgenic tomato and tobacco plants" * Abstract *	1-4,7,8, 11,12,13, 15-17,23, 24,25	C 12 N A 01 H
A	THE PLANT CELL, vol. 1, April 1989, pages 403-413, American Society of Plant Physiologists; J.I. MEDFORD et al.: "Alterations of endogenous cytokinins in transgenic plants using a chimeric isopentenyl transferase gene" * Page 406, left-hand column *	1,15,16, 17	
	-/-		

The present search report has been drawn up for all claims

Place of search	Date of completion of search	Examiner
The Hague	26 October 90	MADDOX A.D.

CATEG RY OF CITED DOCUMENTS

X: particularly relevant if taken alone
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D: document cited in the application
L: document cited for other reasons

&: member of the same patent family, corresponding document

European Patent Office

EUROPEAN SEARCH REPORT

Application Number

EP 90 40 2196

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
A	SCIENCE, vol. 244, 14th April 1989, pages 174-181; P.N. BENFEY et al.: "Regulated genes in transgenic plants" * Page 178, right-hand column: "Cell-specific expression of the EPSP synthase gene construct", paragraph 3 *	23,24	
A	EMBO JOURNAL, vol. 7, no. 9, 1988, pages 2621-2629, IRL Press Ltd, Oxford, GB; T. SCHMÜLLING et al.: "Single genes form Agrobacterium rhizogenes influence plant development" * Page 2622, right-hand column, paragraph 3 *	1,15-17	
A	EP-A-0 329 308 (PALADIN HYBRIDS) * Whole document & AU-D-29 632 (Family member published 03-08-1989)	1-25	
P,A	EP-A-0 344 029 (PLANT GENETIC SYSTEMS) * Whole document *	7,8	
A	EP-A-0 198 288 (ADVANCED GENETIC SCIENCES) * Whole document *	4,10	

TECHNICAL FIELDS SEARCHED (Int. Cl.5)

The present search report has been drawn up for all claims

Place of search	Date of completion of search	Examiner
The Hague	26 October 90	MADDOX A.D.

CATEGORY OF CITED DOCUMENTS

X: particularly relevant if taken alone
Y: particularly relevant if combined with another document of the same catagory
A: technological background
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P: intermediate document
T: theory or principle underlying the invention

E: earlier patent document, but published on, or after the filing date
D: document cited in the application
L: document cited for other reasons

&: member of the same patent family, corresponding document